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(54) **METHODS OF MODIFYING FEEDING BEHAVIOR, COMPOUNDS USEFUL IN SUCH METHODS, AND DNA ENCODING A HYPOTHALAMIC ATYPICAL NEUROPEPTIDE Y/PEPTIDE YY RECEPTOR (Y5)**

VERFAHREN ZUR VERÄNDERUNG DES ESSVERHALTENS, DAFÜR VERWENDBARE VERBINDUNGEN UND DNA, DIE EINEN HYPOTHALAMISCHEN ATYPISCHEN NEUROPEPTID Y/PEPTID YY REZEPTOR (Y5) KODIERT

PROCEDES DE MODIFICATION DU COMPORTEMENT ALIMENTAIRE, COMPOSES UTILES DANS CES PROCEDES, ET ADN CODANT UN RECEPTEUR (Y5) D'UN NEUROPEPTIDE Y/PEPTIDE YY ATYPIQUE HYPOTHALAMIQUE

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- **NATURE, vol. 382, no. 6587, 11 July 1996, LONDON GB, pages 168-171, XP000612078 GERALD, C. ET AL.: "A receptor subtype involved neuropeptide-Y-induced food intake"**

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Description

Background of the Invention

[0001] Throughout this application, various references are referred to within parentheses. Disclosures of these publications in their entireties are hereby incorporated by reference into this application to more fully describe the state of the art to which this invention pertains. Full bibliographic citation for these references may be found at the end of this application, preceding the sequence listing and the claims.

[0002] Neuropeptide Y (NPY) is a member of the pancreatic polypeptide family with widespread distribution throughout the mammalian nervous system. NPY and its relatives (peptide YY or PYY, and pancreatic polypeptide or PP) elicit a broad range of physiological effects through activation of at least five G protein-coupled receptor subtypes known as Y1, Y2, Y3, Y4 (or PP), and the "atypical Y1". The role of NPY as the most powerful stimulant of feeding behavior yet described is thought to occur primarily through activation of the hypothalamic "atypical Y1" receptor. This receptor is unique in that its classification was based solely on feeding behavior data, rather than radioligand binding data, unlike the Y1, Y2, Y3, and Y4 (or PP) receptors, each of which were described previously in both radioligand binding and functional assays. Applicants now report the use of a ^{125}I-PYY-based expression cloning technique to isolate a rat hypothalamic cDNA encoding an "atypical Y1" receptor referred to herein as the Y5 subtype. Applicants also report the isolation and characterization of a Y5 homolog from human hippocampus. Protein sequence analysis reveals that the Y5 receptor belongs to the G protein-coupled receptor superfamily. Both the human and rat homolog display s 42% identity in transmembrane domains with the previously cloned "Y-type" receptors. Rat brain localization studies using in situ hybridization techniques verified the existence of Y5 receptor mRNA in rat hypothalamus. Pharmacological evaluation revealed the following similarities between the Y5 and the "atypical Y1" receptor. 1) Peptides bound to the Y5 receptor with a rank order of potency identical to that described for the feeding response: NPY ≥ NPY_{2-36} = PYY = [Leu^{31}, Pro^{34}] NPY >> NPY_{13-36}. 2) The Y5 receptor was negatively coupled to cAMP accumulation, as had been proposed for the "atypical Y1" receptor. 3) Peptides activated the Y5 receptor with a rank order of potency identical to that described for the feeding response. 4) The reported feeding "modulator" [D-Trp^{32}]NPY bound selectively to the Y5 receptor and subsequently activated the receptor. 5) Both the Y5 and the "atypical Y1" receptors were sensitive to deletions or modifications in the midregion of NPY and related peptide ligands. These data support the identity of the Y5 receptor as the previously described "atypical Y1", and furthermore indicate a role for the Y5 receptor as a potential target in the treatment of obesity, metabolism, and appetite disorders.

[0003] The peptide neurotransmitter neuropeptide Y (NPY) is a 36 amino acid member of the pancreatic polypeptide family with widespread distribution throughout the mammalian nervous system. NPY is considered to be the most powerful stimulant of feeding behavior yet described (Clark et al., 1984; Levine and Morley, 1984; Stanley and Leibowitz, 1984). Direct injection into the hypothalamus of satiated rats, for example, can increase food intake up to 10-fold over a 4-hour period (Stanley et al., 1992). The role of NPY in normal and abnormal eating behavior, and the ability to interfere with NPY-dependent pathways as a means to appetite and weight control, are areas of great interest in pharmacological and pharmaceutical research (Sahu and Kalra, 1993; Dryden et al., 1994). Any credible means of studying or controlling NPY-dependent feeding behavior, however, must necessarily be highly specific as NPY can act through at least 5 pharmacologically defined receptor subtypes to elicit a wide variety of physiological functions (Dumont et al., 1992). It is therefore vital that knowledge of the molecular biology and structural diversity of the individual receptor subtypes be understood as part of a rational drug design approach to develop subtype selective compounds. A brief review of NPY receptor pharmacology is summarized below and also in Table 1.

TABLE 1: Pharmacologically defined receptors for NPY and related pancreatic polypeptides.

[0004] Rank orders of affinity for key peptides (NPY, PYY, PP, [Leu^{31}, Pro^{34}]NPY, NPY_{2-36}, and NPY_{13-36}) are based on previously reported binding and functional data (Schwartz et al., 1990; Wahlestedt et al., 1991; Dumont et al., 1992; Wahlestedt and Reis, 1993). Data for the Y2 receptor were disclosed in U.S. patent application 08/192,288 filed on 2/3/94, currently pending, the foregoing contents of which are hereby incorporated by reference. Data for the Y4 receptor were disclosed in U.S. patent application 08/176,412 filed on 12/28/93, currently pending, the foregoing contents of which are hereby incorporated by reference. Missing peptides in the series reflect a lack of published information.

TABLE 1

Receptor	Affinity (pK_i or pEC_{50})					
	11 to 10	10 to 9	9 to 8	8 to 7	7 to 6	< 6
Y1	NPY PYY [Leu^{31},Pro^{34}] NPY		NPY_{2-36}	NPY_{13-36}	PP	
Y2		PYY NPY NPY_{2-36}	NPY_{13-36}			[Leu^{31}, Pro^{34}] NPY PP
Y3		NPY	[Pro^{34}] NPY	NPY_{13-36} PP		PYY
Y4	PP	PYY [Leu^{31},P ro^{34}] NPY	NPY NPY_{2-36}	NPY_{13-36}		
atypical Y1 (feeding)		PYY NPY NPY_{2-36} [Leu^{31},P ro^{34}] NPY		NPY_{13-36}		

NPY Receptor Pharmacology

[0005] NPY receptor pharmacology has historically been based on structure/activity relationships within the pancreatic polypeptide family. The entire family includes the namesake pancreatic polypeptide (PP), synthesized primarily by endocrine cells in the pancreas; peptide YY (PYY), synthesized primarily by endocrine cells in the gut; and NPY, synthesized primarily in neurons (Michel, 1991; Dumont et al., 1992; Wahlestedt and Reis, 1993). All pancreatic polypeptide family members share a compact structure involving a "PP-fold" and a conserved C-terminal hexapeptide ending in Tyr^{36} (or Y^{36} in the single letter code). The striking conservation of Y^{36} has prompted the reference to the pancreatic polypeptides' receptors as "Y-type" receptors (Wahlestedt et al., 1987), all of which are proposed to function as seven transmembrane-spanning G protein-coupled receptors (Dumont et al., 1992).

[0006] The Y1 receptor recognizes NPY ≥ PYY >> PP (Grundemar et al., 1992). The receptor requires both the N- and the C-terminal regions of the peptides for optimal recognition. Exchange of Gln^{34} in NPY or PYY with the analogous residue from PP (Pro^{34}), however, is well-tolerated. The Y1 receptor has been cloned from a variety of species including human, rat and mouse (Larhammar et al, 1992; Herzog et al, 1992; Eva et al, 1990; Eva et al, 1992). The Y2 receptor recognizes PYY ~ MPY >> PP and is relatively tolerant of N-terminal deletion (Grundemar et al., 1992). The receptor has a strict requirement for structure in the C-terminus (Arg^{33}-Gln^{34}-Arg^{35}-Tyr^{36}-NH_2); exchange of Gln^{34} with Pro^{34}, as in PP, is not well tolerated. The Y2 receptor has recently been cloned (disclosed in US patent application Serial No. 08/192,288, filed February 3, 1994). The Y3 receptor is characterized by a strong preference for NPY over PYY and PP (Wahlestedt et al., 1991). [Pro^{34}]NPY is reasonably well tolerated even though PP, which also contains Pro^{34}, does not bind well to the Y3 receptor. This receptor (Y3) has not yet been cloned. The Y4 receptor (disclosed in U.S. patent application Serial No. 08/176,412, filed December 28, 1993) binds PP > PYY > NPY. Like the Y1, the Y4 requires both the N- and the C-terminal regions of the peptides for optimal recognition (Synaptic Y4 patent). The "atypical Y1" or "feeding" receptor was defined exclusively by injection of several pancreatic polypeptide analogs into the paraventricular nucleus of the rat hypothalamus which stimulated feeding behavior with the following rank order: NPY_{2-36} ≥ NPY ~ PYY ~ [Leu^{31}, Pro^{34}]NPY > NPY_{13-36} (Kalra et al., 1991; Stanley et al., 1992). The profile is similar to that of a Y1-like receptor except for the anomalous ability of NPY_{2-36} to stimulate food intake with potency equivalent or better than that of NPY. A subsequent report in *J. Med. Chem.* by Balasubramaniam and co-workers (1994) showed that feeding can be regulated by [D-Trp^{32}]NPY. While this peptide was presented as an NPY antagonist, the published data at least in part support a stimulatory effect of [D-Trp^{32}]NPY on feeding. [D-Trp^{32}]NPY thereby represents another diagnostic tool for receptor identification. In contrast to other NPY receptor subtypes, the "feeding" receptor has never been characterized for peptide binding affinity in radioligand binding assays and the fact that a single receptor could be responsible for the feeding response has been impossible to validate in the absence of an isolated receptor protein;

the possibility exists, for example, that the feeding response could be a composite profile of Y1 and Y2 subtypes.

[0007] Applicants now report the isolation by expression cloning of a novel Y-type receptor from a rat hypothalamic cDNA library, along with its pharmacological characterization, in situ localization, and human homologues. The data provided link this newly-cloned receptor subtype, from now on referred to as the Y5 subtype, to the "atypical Y1" feeding response. This discovery therefore provides a novel approach, through the use of heterologous expression systems, to develop a subtype selective antagonist for obesity and other indications.

[0008] Applicants further report the isolation of a canine Y5 receptor. In addition, applicants report the discovery of chemical compounds which bind selectively to the Y5 receptor of the present invention and which act as antagonists of the Y5 receptor. Several of the compounds were further shown to inhibit food intake in rats.

[0009] The treatment of disorders or diseases associated with the inhibition of the Y5 receptor subtype, especially diseases caused by eating disorders such as obesity, bulimia nervosa, diabetes, and dislipidimia may be effected by administration of compounds which bind selectively to the Y5 receptor and inhibit the activation of the Y5 receptor. Furthermore, any disease states in which the Y5 receptor subtype is involved, for example, memory loss, epileptic seizures, migraine, sleep disturbance, and pain may also be treated using compounds which bind selectively to the Y5 receptor.

[0010] This invention provides a nucleic acid encoding a mammalian Y5 receptor. This invention also provides a purified Y5 receptor protein. This invention provides a vector comprising the above-described nucleic acid.

[0011] This invention provides a vector which comprises the regulatory elements necessary for expression of DNA in a mammalian cell operatively linked to the DNA encoding the human Y5 receptor as to permit expression thereof designated pcEXV-hY5 (ATCC Accession No. 75943).

[0012] This invention provides a plasmid which comprises the regulatory elements necessary for expression of DNA in a mammalian cell operatively linked to the DNA encoding the rat Y5 receptor as to permit expression thereof designated pcEXV-rY5 (ATCC Accession No. 75944).

[0013] This invention provides a mammalian cell comprising the above-described plasmid or vector.

[0014] This invention provides a nucleic acid probe comprising a nucleic acid of at least 15 nucleotides capable of specifically hybridizing with a unique sequence included within the sequence of a nucleic acid encoding a Y5 receptor.

[0015] This invention provides an antisense oligonucleotide having a sequence capable of specifically hybridizing to mRNA encoding a Y5 receptor so as to prevent translation of the mRNA.

[0016] This invention provides an antibody directed to a Y5 receptor.

[0017] This invention provides a pharmaceutical composition which comprises the antibody above-described and a pharmaceutically acceptable carrier.

[0018] This invention provides a pharmaceutical composition comprising an amount of an antagonist effective to reduce the activity of a human Y5 receptor and a pharmaceutically acceptable carrier.

[0019] This invention provides a pharmaceutical composition comprising an amount of an agonist effective to increase activity of a Y5 receptor and a pharmaceutically acceptable carrier.

[0020] This invention provides the above-described pharmaceutical composition which comprises an amount of the antibody effective to block binding of a ligand to the Y5 receptor and a pharmaceutically acceptable carrier.

[0021] This invention provides a transgenic nonhuman mammal expressing DNA encoding a human Y5 receptor.

[0022] This invention also provides a method for determining whether a chemical compound specifically binds to a Y5 receptor
which comprises contacting host cells or membrane preparations with the chemical compound under conditions suitable for binding, and detecting specific binding of the chemical compound to the Y5 receptor.

[0023] This invention provides a process for determining whether a chemical compound is a Y5 receptor agonist, which comprises contacting host cells or membrane preparations with the chemical compound under conditions permitting activation of the Y5 receptor, and detecting an increase in Y5 receptor activity, so as to thereby determine whether the chemical compound is a Y5 receptor agonist.

[0024] This invention provides a method for determining whether a ligand is a Y5 receptor antagonist which comprises contacting a cell transfected with and expressing DNA encoding a Y5 receptor with the ligand in the presence of a known Y5 receptor agonist, such as PYY or NPY, under conditions permitting the activation of the Y5 receptor, detecting a decrease in Y5 receptor activity, and thereby determining whether the ligand is a Y5 receptor antagonist.

[0025] This invention provides a process involving competitive binding to identify a chemical compound which specifically binds to a Y5 receptor, which comprises separately contacting host cells or membrane preparations with both a chemical compound known to bind specifically to the Y5 receptor and a plurality of chemical compounds not known to bind specifically to the Y5 receptor, and with only the chemical compound known to bind to the Y5 receptor under conditions suitable for binding of compounds, detecting specific binding of the plurality of chemical compounds, a decrease in the binding of the chemical compound known to bind to the Y5 receptor in the presence of the plurality of chemical compounds indicating that a least a chemical compound included in the plurality of chemical compounds binds to the Y5 receptor, and separately detecting the binding of each chemical compound included in the plurality of

compounds to the Y5 receptor.

[0026] This invention provides a method of screening a plurality of chemical compounds not known to bind to a Y5 receptor to identify a compound which specifically binds to the Y5 receptor, which comprises (a) preparing a cell extract from cells transfected with and expressing DNA encoding the Y5 receptor, isolating a membrane fraction from the cell extract, contacting the membrane fraction with a compound known to bind specifically to the Y5 receptor; (b) contacting preparation of step (a) with the plurality of compounds not known to bind specifically to the Y5 receptor, under conditions permitting binding of compounds known to bind the Y5 receptor; (c) determining whether the binding of the compound known to bind to the Y5 receptor is reduced in the presence of the compounds, relative to the binding of the compound in the absence of the plurality of compounds; and if so (d) separately determining the binding to the Y5 receptor of each compound included in the plurality of compounds, so as to thereby identify the compound which specifically binds to the Y5 receptor.

[0027] This invention provides a process for determining whether a chemical compound specifically binds to and activates a Y5 receptor, which comprises contacting host cells or membrane preparations with a plurality of chemical compounds not known to bind and activate the Y5 receptor, under conditions suitable for activation of the Y5 receptor, measuring a second messenger response in the presence and in the absence of the plurality of chemical compounds, a change in the second messenger response in the presence of the plurality of chemical compounds indicating that at least a chemical compound in the plurality of chemical compound activates the Y5 receptor, and separately determining whether each compound included in the plurality of compounds binds to and activates the Y5 receptor.

[0028] This invention provides a process for determining whether a chemical compound is a Y5 receptor antagonist which comprises contacting host cells or membrane preparations with the chemical compound in the presence of a known Y5 receptor agonist under conditions permitting activation of the Y5 receptor and detecting a decrease in Y5 receptor activity, so as to thereby determine whether the chemical compound is a Y5 receptor antagonist.

[0029] This invention provides a process for determining whether a chemical compound specifically binds to and inhibits activation of a Y5 receptor, which comprises separately contacting host cells or membrane preparations with both the chemical compound and a second chemical compound known to activate the Y5 receptor, and with only the second chemical compound, under conditions suitable for activation of the Y5 receptor and measuring a second messenger response in the presence of only the second chemical compound and in the presence of both the second chemical compound and the chemical compound, a smaller change in second messenger response in the presence of both the chemical compound and the second chemical compound indicating that the chemical compound inhibits activation of the Y5 receptor.

[0030] This invention provides a process for determining whether a chemical compound specifically binds to and inhibits activation of a Y5 receptor, which comprises separately contacting host cells or membrane preparations, with both a chemical compound known to activate the Y5 receptor and a plurality of compounds not known to inhibit activation of the Y5 receptor, and with only the chemical compound known to activate the Y5 receptor, under conditions suitable for activation of the Y5 receptor, and measuring a second messenger response in the presence of only the chemical compound known to activate the Y5 receptor and in the presence of both the chemical compound known to activate the Y5 receptor and the plurality of chemical compounds, a smaller change in the second messenger response in the presence of both the chemical compound known to activate the Y5 receptor and the plurality of chemical compounds than in the presence of only the chemical compound known to activate the Y5 receptor indicating that at least a chemical compound included in the plurality of chemical compounds inhibits activation of the Y5 receptor, and separately determining whether each compound included in the plurality of chemical compound specifically binds to and inhibits activation of the Y5 receptor.

[0031] In a separate embodiment of the above-described process, the second messenger response comprises adenylate cyclase activity and the change in second messenger response is a smaller decrease in the level of adenylate cyclase activity in the presence of both the_chemical compound and the second chemical compound, or the chemical compound known to activate the Y5 receptor and the plurality of chemical compounds, than in the presence of only the second chemical compound, or the chemical compound known to activate the Y5 receptor.

[0032] This invention provides a method of screening drugs to identify drugs which act as agonists of a Y5 receptor which comprises contacting a cell transfected with and expressing DNA encoding a Y5 receptor with a plurality of drugs under conditions permitting the activation of a functional Y5 receptor response, determining those drugs which activate such receptor in the cell, and thereby identify drugs which act as Y5 receptor agonists.

[0033] This invention provides a method of screening drugs to identify drugs which act as Y5 receptor antagonists which comprises contacting cells transfected with and expressing DNA encoding a Y5 receptor with a plurality of drugs in the presence of a known Y5 receptor agonist, such as PYY or NPY, under conditions permitting the activation of a functional Y5 receptor response, determining those drugs which inhibit the activation of the receptor in the mammalian cell, and thereby identifying drugs which act as Y5 receptor antagonists.

[0034] This invention provides a method for diagnosing a predisposition to a disorder associated with the activity of a specific human Y5 receptor allele which comprises:

a. obtaining DNA of subjects suffering from the disorder; performing a restriction digest of the DNA with a panel of restriction enzymes; c. electrophoretic-ally separating the resulting DNA fragments on a sizing gel;
d. contacting the resulting gel with a nucleic acid probe capable of specifically hybridizing to DNA encoding a human Y5 receptor and labelled with a detectable marker;
e. detecting labelled bands which have hybridized to the DNA encoding a human Y5 receptor labelled with a detectable marker to create a unique band pattern specific to the DNA of subjects suffering from the disorder; f. preparing DNA obtained for diagnosis by steps a-e; and g. comparing the unique band pattern specific to the DNA of subjects suffering from the disorder from step e and the DNA obtained for diagnosis from step f to determine whether the patterns are the same or different and to diagnose thereby predisposition to the disorder if the patterns are the same.

[0035] This invention provides a method of preparing the purified human, rat or canine Y5 receptor which comprises: a) constructing a vector adapted for expression in a cell which comprises the regulatory elements necessary for the expression of the nucleic acid in the cell operatively linked to the nucleic acid encoding the human, rat or canine Y5 receptor as to permit expression thereof, wherein the cell is selected from a group consisting of bacterial cells, yeast cells, insect cells and mammalian cells; b) inserting the vector of step a) in a suitable host cell; c) incubating the cells of step b) under conditions allowing expression of the human, rat or canine Y5 receptors; d) recovering the receptor so produced; and e) purifying the receptor so recovered, thereby preparing a human, rat or canine Y5 receptor.

Brief Description of the Figures

[0036]

Figure 1 Competitive displacement of ^{125}I-PYY on membranes from rat hypothalamus. Membranes were incubated with ^{125}I-PYY and increasing concentrations of peptide competitors. IC_{50} values corresponding to 50% displacement were determined by nonlinear regression analysis. Data are representative of at least two independent experiments. IC_{50} values for these compounds are listed separately in Table 2.

Figure 2 Competitive displacement of ^{125}I-PYY_{3-36} on membranes from rat hypothalamus. Membranes were incubated with ^{125}I-PYY_{3-36} and increasing concentrations of peptide competitors. IC_{50} values corresponding to 50% displacement were determined by nonlinear regression analysis. Data are representative of at least two independent experiments. IC_{50} values for these compounds are listed separately in Table 2.

Figure 3 Nucleotide sequence of the rat hypothalamic Y5 cDNA clone (Seq. I.D. No 1). Initiation and stop codons are underlined. Only partial 5' and 3' untranslated sequences are shown.

Figure 4 Corresponding amino acid sequence of the rat hypothalamic Y5 cDNA clone (Seq. I.D. No. 2).

Figure 5 Nucleotide sequence of the human hippocampal Y5 cDNA clone (Seq. I.D. No. 3). Initiation and stop codons are underlined. Only partial 5' and 3' untranslated sequences are shown.

Figure 6 Corresponding amino acid sequence of the human hippocampal Y5 cDNA clone(Seq. I.D. No. 4).

Figure 7 A-E. Comparison of coding nucleotide sequences between rat hypothalamic Y5 (top row) and human hippocampal Y5 (bottom row) cDNA clones (84.1% nucleotide identity). F-G. Comparison of deduced amino acid sequence between rat hypothalamic Y5 (top row) and human hippocampal Y5 (Bottom row) cDNA clones (87.2% overall and 98.8% transmembrane domain identities).

Figure 8 Comparison of the human Y5 receptor deduced amino acid sequence with those of the human Y1, Y2, Y4 sequences. Solid bars, the seven putative membrane-spanning domains (TM I-VII). Shading, identities between receptor sequences.

Figure 9 Equilibrium binding of ^{125}I-PYY to membranes from COS-7 cells transiently expressing rat Y5 receptors. Membranes were incubated with ^{125}I-PYY for the times indicated, in the presence or absence of 300 nM human NPY. Specific binding, B, was plotted against time, t, to obtain the maximum number of equilibrium binding sites, B_{max}, and observed association rate, K_{obs}, according to the equation, $B = B_{max} * (1 - e^{-(kobs * t)})$. Binding is shown as the percentage of total equilibrium binding, B_{max}, determined by nonlinear regression analysis. Each point represents a triplicate determination.

Figure 10 Saturable equilibrium binding of ^{125}I-PYY to membranes from COS-7 cells transiently expressing rat Y5 receptors. Membranes were incubated with ^{125}I-PYY ranging in concentration from 0.4 pM to 2.7 nM, in the presence or absence of 300 nM human NPY. Specific binding, B, was plotted against the free ^{125}I-PYY concentration, [L], to obtain the maximum number of saturable binding sites, B_{max}, and the ^{125}I-PYY equilibrium dissociation constant, K_d, according to the binding isotherm, $B = B_{max}[L]/([L] + K_d)$. Specific binding is shown. Data are representative of three independent experiments, with each point measured in triplicate.

Figure 11 Competitive displacement of ^{125}I-PYY from COS-7 cells transiently expressing rat Y5 receptors. Membranes were incubated with ^{125}I-PYY and increasing concentrations of peptide competitors. IC_{50} values corresponding to 50% displacement were determined by nonlinear regression analysis and converted to K_i values according to the equation, $K_i=IC_{50}/(1 + [L]/K_d)$, where [L] is the ^{125}I-PYY concentration and K_d is the equilibrium dissociation constant of ^{125}I-PYY. Data are representative of at least two independent experiments. Rank orders of affinity for these and other compounds are listed separately in Table 4.

Figure 12 Inhibition of forskolin-stimulated cAMP accumulation in intact 293 cells stably expressing rat Y5 receptors. Functional data were derived from radioimmunoassay of CAMP in 293 cells stimulated with 10 µM forskolin over a 5 minute period. Rat/human NPY was tested for agonist activity at concentrations ranging from 0.03 pM to 0.3 µM over the same period. The EC_{50} value corresponding to 50% maximal activity was determined by nonlinear regression analysis. The data shown are representative of three independent experiments.

Figure 13 Schematic diagrams of coronal sections through the rat brain, illustrating the distribution of NPY Y5 receptor mRNA, as visualized microscopically in sections dipped in liquid emulsion. The sections are arranged from rostral (A) to caudal (H). Differences in silver grain density over individual neurons in a given area are indicated by the hatching gradient. The full definitions for the abbreviations are as follows:

Aco = anterior cortical amygdaloid nucleus;
AD = anterodorsal thalamic nucleus;
APT = anterior pretectal nucleus;
Arc = arcuate hypothalamic nucleus;
BLA = basolateral amygdaloid nucleus anterior;
CA3 = field CA3 of Ammon's horn, hippocampus;
CeA = central amygdaloid nucleus;
Cg = cingulate cortex;
CL = centrolateral thalamic nucleus;
CM = central medial thalamic nucleus
DG = dentate gyrus, hippocampus;
DMH = dorsomedial hypothalamic nucleus;
DR = dorsal raphe;
GiA = gigantocellular reticular nucleus, alpha;
HDB = nucleus horizontal limb diagonal band;
InG = intermediate gray layer superior colliculus;
LC = locus coeruleus;
LH = lateral hypothalamic area;
MePV = medial amygdaloid nucleus, posteroventral;
MVe = medial vestibular nucleus;
MHb = medial habenular nucleus;
MPN = medial preoptic nucleus;
PAG = periaqueductal gray;
PaS = parasubiculum;
PC = paracentral thalamic nucleus;
PCRtA = parvocellular reticular nucleus, alpha;
Pe = periventricular hypothalamic nucleus;
PrS = presubiculum;
PN = pontine nuclei;
PVH = paraventricular hypothalamic nucleus;
PVHmp = paraventricular hypothalamic nucleus, medial parvicellular part
PVT = paraventricular thalamic nucleus;

Re = reunions thalamic nucleus;
RLi = rostral linear nucleus raphe;
RSG = retrosplenial cortex;
SCN = suprachiasmatic nucleus;
SNc = substantia nigra, pars compacta; and
SON = supraoptic nucleus.

Figure 14 Partial Nucleotide sequence of the canine Y5 cDNA clone beginning immediately upstream of TM III to the stop codon (underlined), (Seq. I.D. No 5). Only partial 3' untranslated sequence is shown.

Figure 15 Corresponding amino acid sequence of the canine Y5 cDNA clone (Seq. I.D. No. 6).

Figure 16 A. Northern blot analysis of various rat tissues. B. Northern blot analysis of various human brain areas: amygdala, caudate nucleus, corpus callosum, hippocampus, whole brain, substantia nigra, subthalamic nucleus, and thalamus. C. Northern blot analysis of various additional human brain areas: cerebellum, cerebral cortex, medula, spinal cord, occipital lobe, frontal lobe, temporal lobe, and putamen. Hybridization was done under conditions of high stringency, as described in Experimental Details.

Figure 17 Southern blot analysis of human or rat genomic DNA encoding the Y5 receptor subtype. Hybridization was done under conditions of high stringency, as described in Experimental Details.

Figure 18 Time course for equilibrium binding of ^{125}I-Leu^{31},Pro^{34}-PYY to the rat Y5 receptor. Membranes were incubated with 0.08 nM radioligand at room temperature for the length of time indicated in binding buffer containing either 10 mM Na+ or 138 mM Na+.

Figure 19 Guanine Nucleotide Modulation of Y5 Peptide Binding. Human or rat Y5 receptors transiently expressed in COS-7 cell membranes, or human Y5 receptors stably expressed in LM(tk-) cell membranes, were incubated with 0.08 nM ^{125}I-PYY and increasing concentrations of Gpp(NH)p as indicated under standard binding assay conditions. Radioligand binding is reported as cpm, efficiency = 0.8. For the human Y5 in LM(tk-) (0.007 mg membrane protein/sample), the maximum Δ cpm = -2343. Given a specific activity of 2200 Ci/mmol, the change in radioligand binding is therefore calculated to be -0.6 fmol/0.007 mg protein = -85 fmol/mg membrane protein.

Figure 20 NPY-Dependent Inhibition of Forskolin Stimulated CAMP Accumulation by Cloned Y5 Receptors. Intact cells stably transfected with human or rat Y5 receptors were incubated with forskolin plus a range of human NPY concentrations as indicated. A representative experiment is shown for each receptor system ($n \geq 2$).

Figure 21 Calcium Mobilization: Fura-2 Assay. Cloned human Y-type receptors in the host cells indicated were screened for intracellular calcium mobilization in response to NPY and related peptides. Representative calcium transients are shown for each receptor system.

Figure 22 Illustrates the structure of a compound which binds selectively to the human and rat Y5 receptors.

Detailed Description of the Invention

[0037] Throughout this application, the following standard abbreviations are used to indicate specific nucleotide bases:

C=cytosine A=adenine
T=thymine G=guanine

[0038] Furthermore, the term "agonist" is used throughout this application to indicate any peptide or non-peptidyl compound which increases the activity of any of the receptors of the subject invention. The term "antagonist" is used throughout this application to indicate any peptide or non-peptidyl compound which decreases the activity of any of the receptors of the subject invention.

[0039] The activity of a G-protein coupled receptor such as a Y5 receptor may be measured using any of a variety of appropriate functional assays in which activation of the receptor in question results in an observable change in the level of some second messenger system, including but not limited to adenylate cyclase, calcium mobilization, inositol phospholipid hydrolysis or guanylyl cyclase.

[0040] This invention provides a nucleic acid encoding a Y5 receptor. In an embodiment, the Y5 receptor is a vertebrate or a mammalian Y5 receptor. In an embodiment, the nucleic acid encodes a mammalian Y5 receptor wherein the nucleic acid contains a nucleotide sequence encoding the amino acid sequence shown in Figures 4, 6 and 15. In another embodiment, the Y5 receptor has substantially the same amino acid sequence as described in Figure 4. In another embodiment, the Y5 receptor has substantially the same amino acid sequence as described in Figure 6.

[0041] This invention provides a nucleic acid, wherein the nucleic acid encodes a Y5 receptor characterized by an amino acid sequence in each of the transmembrane regions I-VII which is identical to the amino acid sequence in the corresponding transmembrane region of the human Y5 receptor shown in Figure 8.

[0042] This invention provides the above-described isolated nucleic acid, wherein the nucleic acid is DNA. In an embodiment, the DNA is cDNA. In another embodiment, the DNA is genomic DNA. In still another embodiment, the nucleic acid is RNA, wherein the RNA is preferably mRNA. In a separate embodiment, the nucleic acid encodes a human Y5 receptor. In an embodiment, the human Y5 receptor has the amino acid sequence as described in Figure 6.

[0043] This invention further provides DNA which is degenerate with any of the DNA shown in Figures 3, 5 and 14, which DNA encode Y5 receptors having the amino acid sequences shown in Figures 4, 6, and 15, respectively.

[0044] This invention also encompasses DNAs and cDNAs which encode amino acid sequences which differ from those of Y5 receptor, but which should not produce phenotypic changes. Alternatively, this invention also encompasses DNAs and cDNAs which hybridize to the DNA and cDNA of the subject invention. Hybridization methods are well known to those of skill in the art.

[0045] The DNA molecules of the subject invention also include DNA coding for polypeptide analogs, fragments or derivatives of antigenic polypeptides which differ from naturally-occurring forms in terms of the identity or location of one or more amino acid residues (deletion analogs containing less than all of the residues specified for the protein, substitution analogs wherein one or more residues specified are replaced by other residues and addition analogs where in one or more amino acid residues is added to a terminal or medial portion of the polypeptides) and which share some or all properties of naturally-occurring forms. These nucleic acids include: the incorporation of codons "preferred" for expression by selected non-mammalian hosts; the provision of sites for cleavage by restriction endonuclease enzymes; and the provision of additional initial, terminal or intermediate DNA sequences that facilitate construction of readily expressed vectors.

[0046] The nucleic acids described and claimed herein are useful for the information which they provide concerning the amino acid sequence of the polypeptide and as products for the large scale synthesis of the polypeptide by a variety of recombinant techniques. The nucleic acid is useful for generating new cloning and expression vectors, transformed and transfected prokaryotic and eukaryotic host cells, and new and useful methods for cultured growth of such host cells capable of expression of the polypeptide and related products.

[0047] In a separate embodiment, the nucleic acid encodes a rat Y5 receptor. In another embodiment, the rat Y5 receptor has the amino acid sequence shown in Figure 4. In another embodiment, the nucleic acid encodes a canine Y5 receptor. In a further embodiment, the canine Y5 receptor has the amino acid sequence as shown in Figure 15.

[0048] This invention also provides a purified Y5 receptor protein. In separate embodiments, the Y5 protein may be a human, a rat, or a canine protein.

[0049] This invention provides a vector comprising the above-described nucleic acid.

[0050] Vectors which comprise the isolated nucleic acid described hereinabove also are provided. Suitable vectors comprise, but are not limited-to, a plasmid or a virus. These vectors may be transformed into a suitable host cell to form a host cell vector system for the production of a polypeptide having the biological activity of a Y5 receptor.

[0051] This invention provides the above-described vector adapted for expression in a host cell which comprises the regulatory elements necessary for expression of the nucleic acid in the host cell operatively linked to the nucleic acid encoding a Y5 receptor so as to permit expression thereof.

[0052] This invention provides the above-described vector adapted for expression in a host cell, wherein the host cell is a bacterial, yeast, insect or mammalian cell. In an embodiment, the host cell is non-neuronal in origin. In a further embodiment, the cell is a COS-7 cell, a 293 human embryonic kidney cell, a NIH-3T3 cell or an LM(tk-) cell. In a still further embodiment, the insect cell is a Sf9 cell or a Sf21 cell.

[0053] This invention provides the above-described vector which is a baculovirus or a plasmid.

[0054] This invention provides a membrane preparation isolated from the host cells above-described, wherein the host cell does not naturally express a Y5 receptor.

[0055] In an embodiment, the vector is adapted for expression in a mammalian cell which comprises the regulatory elements necessary for expression of the DNA in the mammalian cell operatively linked to the DNA encoding the mammalian Y5 receptor as to permit expression thereof.

[0056] In a further embodiment, the vector is adapted for expression in a mammalian cell which comprises the regulatory elements necessary for expression of the DNA in the mammalian cell operatively linked to the DNA encoding the human Y5 receptor as to permit expression thereof.

[0057] In a still further embodiment, the plasmid is adapted for expression in a mammalian cell which comprises the

regulatory elements necessary for expression of the DNA in the mammalian cell operatively linked to the DNA encoding the rat Y5 receptor as to permit expression thereof.

[0058] This invention provides the above-described plasmid adapted for expression in a mammalian cell which comprises the regulatory elements necessary for expression of DNA in a mammalian cell operatively linked to the DNA encoding the mammalian Y5 receptor as to permit expression thereof.

[0059] This invention provides a plasmid which comprises the regulatory elements necessary for expression of DNA in a mammalian cell operatively linked to the DNA encoding the human Y5 receptor as to permit expression thereof designated pcEXV-hY5 (ATCC-Accession No. 75943).

[0060] This plasmid (pcEXV-hY5) was deposited on November 4, 1994 with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland 20852, U.S.A. under the provisions of the Budapest Treaty for the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure and was accorded ATCC Accession No. 75943.

[0061] This invention provides a plasmid which comprises the regulatory elements necessary for expression of DNA in a mammalian cell operatively linked to the DNA encoding the rat Y5 receptor as to permit expression thereof designated pcEXV-rY5 (ATCC Accession No. 75944).

[0062] This plasmid (pcEXV-rY5) was deposited on November 4, 1994 with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland 20852, U.S.A. under the provisions of the Budapest Treaty for the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure and was accorded ATCC Accession No. CRL 75944. This invention provides a plasmid designated Y5-bd-5 (ATCC Accession No. _____). This invention also provides a plasmid designated Y5-bd-8 (ATCC Accession No. _____). These plasmids were deposited - on December 1, 1995 with the American Type Culture Collection (ATCC) 12301 Parklawn Drive, Rockville, Maryland 20852, U.S.A. under the provisions of the Budapest Treaty for the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure and was accorded ATCC Accession Nos. _____ and _____, respectively

[0063] This invention provides a baculovirus designated hY5-BB3 (ATCC Accession No. _____) This baculovirus was deposited on Novmeber 15, 1995 with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland 20852, U.S.A. under the provisions of the Budapest Treaty for the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure and was accorded ATCC Accession No. _____.

[0064] This invention provides a mammalian cell comprising the above-described plasmid or vector. In an embodiment, the mammalian cell is a COS-7 cell.

[0065] In another embodiment, the mammalian cell is a 293 human embryonic kidney cell designated 293-rY5-14 (ATCC Accession No. CRL 11757).

[0066] This cell (293-rY5-14) was deposited on November 4, 1994 with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland 20852, U.S.A. under the provisions of the Budapest Treaty for the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure and was accorded ATCC Accession No. CRL 11757.

[0067] In a further embodiment, the mammalian cell is a mouse fibroblast (tk-) cell, containing the plasmid pcEXV-hY5 and designated L-hY5-7 (ATCC Accesssion No. CRL-11995). In another embodiment, the mammalian cell is a mouse embryonic NIH-3T3 cell containing the plasmid pcEXV-hY5 and designated N-hY5-8 (ATCC Accession No. CRL-11994). These cells were deposited on November 15, 1995 with the American Type Culture Collection (ATCC) 12301 Parklawn Drive, Rocville, Maryland 20852, U.S.A. under the provisions of the Budapest Treaty for the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure, and were accorded ATCC Accession Nos. CRL-11995 and CRL-11994, respectively.

[0068] This invention provides a nucleic acid probe comprising a nucleic acid of at least 15 nucleotides capable of specifically hybridizing with a unique sequence included within the sequence of a nucleic acid encoding a Y5 receptor. In an embodiment, the nucleic acid is DNA.

[0069] This nucleic acid produced can either be DNA or RNA. As used herein, the phrase "specifically hybridizing" means the ability of a nucleic acid to recognize a nucleic acid sequence complementary to its own and to form double-helical segments through hydrogen bonding between complementary base pairs.

[0070] This nucleic acid of at least 15 nucleotides capable of specifically hybridizing with a sequence of a nucleic acid encoding the human Y5 receptors can be used as a probe. Nucleic acid probe technology is well known to those skilled in the art who will readily appreciate that such probes may vary greatly in length and may be labeled with a detectable label, such as a radioisotope or fluorescent dye, to facilitate detection of the probe. DNA probes may be produced by insertion of a DNA which encodes the Y5 receptor into suitable vectors, such as plasmids or bacteriophages, followed by transforming into suitable bacterial host cells, replication in the transformed bacterial host cells and harvesting of the DNA probes, using methods well known in the art. Alternatively, probes may be generated chemically from DNA synthesizers.

[0071] RNA probes may be generated by inserting the DNA which encodes the Y5 receptor downstream of a bacteriophage promoter such as T3, T7 or SP6. Large amounts of RNA probe may be produced by incubating the labeled nucleotides with the linearized fragment where it contains an upstream promoter in the presence of the appropriate RNA polymerase.

[0072] This invention also provides a nucleic acid of at least 15 nucleotides capable of specifically hybridizing with a sequence of a nucleic acid which is complementary to the mammalian nucleic acid encoding a Y5 receptor. This nucleic acid may either be DNA or RNA.

[0073] This invention provides an antisense oligonucleotide having a sequence capable of specifically hybridizing to mRNA encoding a Y5 receptor so as to prevent translation of the mRNA.

[0074] This invention provides an antisense oligonucleotide having a sequence capable of specifically hybridizing to the genomic DNA of a Y5 receptor.

[0075] This invention provides an antisense oligonucleotide of Y5 receptor comprising chemical analogues of nucleotides.

[0076] This invention provides an antibody capable of binding to a Y5 receptor. This invention also provides an antibody capable of competitively inhibiting the binding to a Y5 receptor of the above-described antibody. In an embodiment, the antibody is a monoclonal antibody.

[0077] This invention provides a monoclonal antibody which is directed to an epitope of a human Y5 receptor present on the surface of a Y5 receptor expressing cell.

[0078] This invention provides a pharmaceutical composition which comprises the antibody above-described and a pharmaceutically acceptable carrier.

[0079] This invention provides the above-described pharmaceutical composition which comprises an amount of the antibody effective to block binding of a ligand to the Y5 receptor and a pharmaceutically acceptable carrier.

[0080] As used herein, "pharmaceutically acceptable carriers" means any of the standard pharmaceutically acceptable carriers. Examples include, but are not limited-to, phosphate buffered saline, physiological saline, water and emulsions, such as oil/water emulsions.

[0081] Animal model systems which elucidate the physiological and behavioral roles of Y5 receptor are produced by creating transgenic animals in which the activity of the Y5 receptor is either increased or decreased, or the amino acid sequence of the expressed Y5 receptor is altered, by a variety of techniques. Examples of these techniques include, but are not limited to: 1) Insertion of normal or mutant versions of DNA encoding a Y5 receptor, by microinjection, electroporation, retroviral transfection or other means well known to those skilled in the art, into appropriate fertilized embryos in order to produce a transgenic animal or 2) Homologous recombination of mutant or normal, human or animal versions of these genes with the native gene locus in transgenic animals to alter the regulation of expression or the structure of these Y5 receptor sequences. The technique of homologous recombination is well known in the art. It replaces the native gene with the inserted gene and so is useful for producing an animal that cannot express native Y5 receptors but does express, for example, an inserted mutant Y5 receptor, which has replaced the native Y5 receptor in the animal's genome by recombination, resulting in underexpression of the transporter. Microinjection adds genes to the genome, but does not remove them, and so is useful for producing an animal which expresses its own and added Y5 receptors, resulting in overexpression of the Y5 receptors.

[0082] One means available for producing a transgenic animal, with a mouse as an example, is as follows: Female mice are mated, and the resulting fertilized eggs are dissected out of their oviducts. The eggs are stored in an appropriate medium such as M2 medium. DNA or cDNA encoding a Y5 receptor is purified from a vector by methods well known in the art. Inducible promoters may be fused with the coding region of the DNA to provide an experimental means to regulate expression of the trans-gene. Alternatively or in addition, tissue specific regulatory elements may be fused with the coding region to permit tissue-specific expression of the trans-gene. The DNA, in an appropriately buffered solution, is put into a microinjection needle (which may be made from capillary tubing using a pipet puller) and the egg to be injected is put in a depression slide. The needle is inserted into the pronucleus of the egg, and the DNA solution is injected. The injected egg is then transferred into the oviduct of a pseudopregnant mouse (a mouse stimulated by the appropriate hormones to maintain pregnancy but which is not actually pregnant), where it proceeds to the uterus, implants, and develops to term. As noted above, microinjection is not the only method for inserting DNA into the egg cell, and is used here only for exemplary purposes.

[0083] This invention also provides a method for determining whether a chemical compound specifically binds to a Y5 receptor which comprises contacting host cells or the membrane preparation with the chemical compound under conditions suitable for binding, and detecting specific binding of the chemical compound to the Y5 receptor.

[0084] This invention provides a process involving competitive binding for identifying a chemical compound which specifically binds to a Y5 receptor, which comprises separately contacting host cells or membrane preparations with both the chemical compound and a second chemical compound known to bind to the Y5 receptor, and with only the second chemical compound, under conditions suitable for binding of compounds, and detecting specific binding of the chemical compound to the Y5 receptor, a decrease in binding of the second chemical compound to the Y5 receptor in

the presence of the chemical compound indicating that the chemical compound binds to the Y5 receptor.

[0085] This invention provides a method for determining whether a ligand can specifically bind to a Y5 receptor which comprises contacting a cell transfected with and expressing DNA encoding the Y5 receptor with the ligand under conditions permitting binding of ligands to such receptor, detecting the presence of any such ligand specifically bound to the Y5 receptor, and thereby determining whether the ligand specifically binds to the human Y5 receptor, such Y5 receptor having substantially the same amino acid sequence shown in Figure 6.

[0086] This invention provides a method for determining whether a ligand can specifically bind to a Y5 receptor which comprises contacting a cell transfected with and expressing DNA encoding the Y5 receptor with the ligand under conditions permitting binding of ligands to such receptor, detecting the presence of any such ligand specifically bound to the Y5 receptor, and thereby determining whether the ligand specifically binds to the Y5 receptor, such Y5 receptor being characterized by an amino acid sequence in the transmembrane region having 60% homology or higher to the amino acid sequence in the transmembrane region of the Y5 receptor shown in Figure 6.

[0087] This invention provides a method for determining whether a ligand can specifically bind to a Y5 receptor which comprises preparing a cell extract from cells transfected with and expressing DNA encoding the Y5 receptor, isolating a membrane fraction from the cell extract, contacting the membrane fraction with the ligand under conditions permitting binding of ligands to such receptor, detecting the presence of the ligand specifically bound to the Y5 receptor, and thereby determining whether the ligand specifically binds to the Y5 receptor.

[0088] In separate embodiments of the above-described methods, the Y5 receptor may be a human Y5 receptor, a rat Y5 receptor, or a canine Y5 receptor.

[0089] This invention provides a method for determining whether a ligand can specifically bind to a Y5 receptor which comprises preparing a cell extract from cells transfected with and expressing DNA encoding the Y5 receptor, isolating a membrane fraction from the cell extract, contacting the membrane fraction with the ligand under conditions permitting binding of ligands to the human Y5 receptor, detecting the presence of the ligand specifically bound to the Y5 receptor, and thereby determining whether the ligand can specifically bind to the Y5 receptor.

[0090] This invention provides a method for determining whether a ligand can specifically bind to a Y5 receptor which comprises preparing a cell extract from cells transfected with and expressing DNA encoding the Y5 receptor, isolating a membrane fraction from the cell extract, contacting the membrane fraction with the ligand under conditions permitting binding of ligands to the Y5 receptor, detecting the presence of the ligand specifically bound to the Y5 receptor, and thereby determining whether the ligand can specifically bind to the Y5 receptor, such Y5 receptor having substantially the same amino acid sequence shown in Figure 6.

[0091] This invention provides a method for determining whether a ligand can specifically bind to a Y5 receptor which comprises preparing a cell extract from cells transfected with and expressing DNA encoding the Y5 receptor, isolating a membrane fraction from the cell extract, contacting the membrane fraction with the ligand under conditions permitting binding of ligands to the Y5 receptor, detecting the presence of the ligand specifically bound to the Y5 receptor, and thereby determining whether the ligand can specifically bind to the Y5 receptor, such Y5 receptor being characterized by an amino acid sequence in the transmembrane region having 60% homology or higher to the amino acid sequence in the transmembrane region of the Y5 receptor shown in Figure 6.

[0092] In separate embodiments of the above-described methods, the Y5 receptor may be a human Y5 receptor, a rat Y5 receptor, or a canine Y5 receptor. In one embodiment of the above-described methods, the ligand is not previously known.

[0093] This invention provides a process for determining whether a chemical compound is a Y5 receptor agonist, which comprises contacting host cells or membrane preparations with the chemical compound under conditions permitting activation of the Y5 receptor, and detecting an increase in Y5 receptor activity, so as to thereby determine whether the chemical compound is a Y5 receptor agonist.

[0094] This invention provides a process for determining whether a chemical compound specifically binds to and activates a Y5 receptor, which comprises contacting host cells or membrane preparations, with the chemical compound under conditions suitable for activation of the Y5 receptor, and measuring a second messenger response in the presence and in the absence of the chemical compound, a change in second messenger response in the presence of the chemical compound indicating that the chemical compound activates the Y5 receptor.

[0095] This invention provides a method for determining whether a ligand is a Y5 receptor agonist which comprises preparing a cell extract from cells transfected with and expressing DNA encoding the Y5 receptor, isolating a membrane fraction from the cell extract, contacting the membrane fraction with the ligand under conditions permitting the activation of the Y5 receptor, and detecting an increase in Y5 receptor activity, so as to thereby determine whether the ligand is a Y5 receptor agonist.

[0096] This invention provides a method for determining whether a ligand is a Y5 receptor antagonist which comprises contacting a cell transfected with and expressing DNA encoding a Y5 receptor with the ligand in the presence of a known Y5 receptor agonist, such as PYY or NPY, under conditions permitting the activation of a functional Y5 receptor response, detecting a decrease in Y5 receptor activity, and thereby determining whether the ligand is a Y5 receptor

antagonist.

[0097] This invention provides a method for determining whether a ligand is a Y5 receptor antagonist which comprises contacting a cell transfected with and expressing DNA encoding a Y5 receptor with the ligand in the presence of a known Y5 receptor agonist, such as PYY or NPY, under conditions permitting the activation of the Y5 receptor, detecting a decrease in Y5 receptor activity, and thereby determining whether the ligand is a Y5 receptor antagonist.

[0098] This invention provides a method for determining whether a ligand is a Y5 receptor antagonist which comprises preparing a cell extract from cells transfected with and expressing DNA encoding the Y5 receptor, isolating a membrane fraction from the cell extract, contacting the membrane fraction with the ligand in the presence of a known Y5 receptor agonist, such as PYY, under conditions permitting the activation of the Y5 receptor, and detecting a decrease in Y5 receptor activity, so as to thereby determine whether the ligand is a Y5 receptor antagonist.

[0099] In separate embodiments of the above-described methods the Y5 receptor is a human Y5 receptor, a rat Y5 receptor, or a canine Y5 receptor.

[0100] In an embodiment of the above-described methods, the cell is non-neuronal in origin. In a further embodiment, the non-neuronal cell is a COS-7 cell, 293 human embryonic kidney cell, NIH-3T3 cell or LM(tk-) cell.

[0101] In one embodiment of the above-described methods, the ligand is not previously known.

[0102] This invention provides a Y5 receptor agonist detected by the above-described method. This invention provides a Y5 receptor antagonist detected by the above-described method.

[0103] This invention provides a method of screening a plurality of chemical compounds not known to bind to a Y5 receptor to identify a compound which specifically binds to the Y5 receptor, which comprises (a) contacting a cell transfected with and expressing DNA encoding the Y5 receptor with a compound known to bind specifically to the Y5 receptor; (b) contacting the preparation of step (a) with the plurality of compounds not known to bind specifically to the Y5 receptor, under conditions permitting binding of compounds known to bind the Y5 receptor; (c) determining whether the binding of the compound known to bind to the Y5 receptor is reduced in the presence of the compounds, relative to the binding of the compound in the absence of the plurality of compounds; and if so (d) separately determining the binding to the Y5 receptor of each compound included in the plurality of compounds, so as to thereby identify the compound which specifically binds to the Y5 receptor.

[0104] This invention provides a process involving competitive binding to identify a chemical compound which specifically binds to a Y5 receptor, which comprises separately contacting host cells or membrane preparations with both a chemical compound known to bind specifically to the Y5 receptor and a plurality of chemical compounds not known to bind specifically to the Y5 receptor, and with only the chemical compound known to bind to the Y5 receptor, under conditions suitable for binding of compounds, detecting specific binding of the plurality of chemical compounds, a decrease in the binding of the chemical compound known to bind the Y5 receptor in the presence of the plurality of chemical compounds indicating that at least a chemical compound included in the plurality of chemical compounds binds to the Y5 receptor, and separately detecting the binding of each chemical compound included in the plurality of compounds to the Y5 receptor.

[0105] This invention provides a process for determining whether a chemical compound specifically binds to and activates a Y5 receptor, which comprises contacting host cells or membrane preparations with a plurality of chemical compounds not known to bind and activate the Y5 receptor, under conditions suitable for activation of the Y5 receptor, measuring a second messenger response in the presence and in the absence of the plurality of chemical compounds, a change in the second messenger response in the presence of the plurality of chemical compounds indicating that at least a chemical compound in the plurality of chemical compounds activates the Y5 receptor, and separately determining whether each compound included in the plurality of compounds binds to and activates the Y5 receptor.

[0106] In one embodiment, the second messenger response comprises adenylate cyclase activity, and the change in second messenger response is a decrease in adenylate cyclase activity. In a further embodiment, the second messenger response comprises intracellular calcium concentration, and the change in second messenger response is an increase in intracellular calcium concentration.

[0107] This invention provides a process for determining whether a chemical compound is a Y5 receptor antagonist, which comprises contacting host cells or membrane preparations, with the chemical compound in the presence of a known Y5 receptor agonist, under conditions permitting activation of the Y5 receptor, and detecting a decrease in Y5 receptor activity, so as to thereby determine whether the chemical compound is a Y5 receptor antagonist.

[0108] This invention provides a process for determining whether a chemical compound specifically binds to and inhibits activation of a Y5 receptor, which comprises separately contacting host cells or membrane preparations with both the chemical compound and a second chemical compound known to activate the Y5 receptor, and with only the second chemical compound, under conditions suitable for activation of the Y5 receptor, and measuring a second messenger response in the presence of only the second chemical compound and in the presence of both the second chemical compound and the chemical compound, a smaller change in second messenger response in the presence of both the chemical compound and the second chemical compound indicating that the chemical compound inhibits activation of the Y5 receptor.

[0109] This invention provides a process for determining whether a chemical compound specifically binds to and inhibits activation of a Y5 receptor, which comprises separately contacting host cells or membrane preparations, with both a chemical compound known to activate the Y5 receptor and a plurality of compounds not known to inhibit activation of the Y5 receptor, and with only the chemical compound known to activate the Y5 receptor, under conditions suitable for activation of the Y5 receptor, and measuring a second messenger response in the presence of only the chemical compound known to activate the Y5 receptor and in the presence of both the chemical compound known to activate the Y5 receptor and the plurality of chemical compounds, a smaller change in the second messenger response in the presence of both the chemical compound known to activate the Y5 receptor and the plurality of chemical compounds than in the presence of only the chemical compound known to activate the Y5 receptor indicating that at least a chemical compound included in the plurality of chemical compounds inhibits activation of the Y5 receptor, and separately determining whether each compound included in the plurality of chemical compounds specifically binds to and inhibits activation of the Y5 receptor.

[0110] In separate embodiments of the above-described process, the Y5 receptor is a human Y5 receptor, a rat Y5 receptor, or a canine Y5 receptor. In an embodiment, the cell is a mammalian cell. In a further embodiment, the cell is non-neuronal in origin. In a further embodiment, the cell is a COS-7 cell, a 293 human embryonic kidney cell, a LM (tk-) cell, or an MIH-3T3 cell.

[0111] In separate embodiments of the above-described process, the second messenger response comprises adenylate cyclase activity, and the change in second messenger response is a smaller decrease in the level of adenylate cyclase activity in the presence of both the chemical compound and the second chemical compound, or the chemical compound known to activate the Y5 receptor and the plurality of chemical compounds than in the presence of only the second chemical compound, or the chemical compound known to activate the Y5 receptor.

[0112] In a further embodiment, the second messenger response comprises intracellular calcium concentration, and the change in second messenger response is a smaller increase in intracellular calcium concentration in the presence of both the chemical and the second chemical compound, or the chemical compound known to activate the Y5 receptor and the plurality of chemical compounds, than in the presence of only the second chemical compound, or the chemical compound known to activate the Y5 receptor:

[0113] This invention provides a method of screening drugs to identify drugs which specifically bind to a human Y5 receptor on the surface of a cell which comprises contacting a cell transfected with and expressing DNA encoding a human Y5 receptor with a plurality of drugs under conditions permitting binding of drugs to the human Y5 receptor, determining those drugs which specifically bind to the transfected cell, and thereby identifying drugs which specifically bind to the human Y5 receptor.

[0114] This invention provides a method of screening drugs to identify drugs which act as agonists of a Y5 receptor which comprises contacting a cell transfected with and expressing DNA encoding a Y5 receptor with a plurality of drugs under conditions permitting the activation of a functional Y5 receptor response, determining those drugs which activate such receptor in the cell, and thereby identify drugs which act as Y5 receptor agonists.

[0115] This invention provides a method of screening drugs to identify drugs which act as agonists of a human Y5 receptor which comprises contacting a cell transfected with and expressing DNA encoding a human Y5 receptor with a plurality of drugs under conditions permitting the activation of a functional human Y5 receptor response, determining those drugs which activate such receptor in the cell, and thereby identify drugs which act as human Y5 receptor agonists.

[0116] This invention provides a method of screening drugs to identify drugs which act as Y5 receptor antagonists which comprises contacting cells transfected with and expressing DNA encoding a Y5 receptor with a plurality of drugs in the presence of a known Y5 receptor agonist, such as PYY or NPY, under conditions permitting the activation of a functional Y5 receptor response, determining those drugs which inhibit the activation of the receptor in the mammalian cell, and thereby identifying drugs which act as Y5 receptor antagonists.

[0117] This invention provides a method of screening drugs to identify drugs which act as human Y5 receptor antagonists which comprises contacting cells transfected with and expressing DNA encoding a human Y5 receptor with a plurality of drugs in the presence of a known human Y5 receptor agonist, such as PYY or NPY, under conditions permitting the activation of a functional human Y5 receptor response, determining those drugs which inhibit the activation of the receptor in the mammalian cell, and thereby identifying drugs which act as human Y5 receptor antagonists. In an embodiment, the cell is non-neuronal in origin. In a further embodiment, the cell is a Cos-7 cell, a 293 human embryonic kidney cell, an LM(tk-) cell or an NIH-3T3 cell.

[0118] This invention provides a pharmaceutical composition comprising a drug identified by the above-described method and a pharmaceutically acceptable carrier.

[0119] This invention provides a method of detecting expression of Y5 receptor by detecting the presence of mRNA coding for the Y5 receptor which comprises obtaining total mRNA from the cell and contacting the mRNA so obtained with the above-described nucleic acid probe under hybridizing conditions, detecting the presence of mRNA hybridized to the probe, and thereby detecting the expression of the Y5 receptor by the cell.

[0120] This invention provides a method of treating an abnormality in a subject, wherein the abnormality is alleviated by the inhibition of a Y5 receptor which comprises administering to a subject an effective amount of the above-described pharmaceutical composition effective to inhibit the Y5 receptor by the subject.

[0121] This invention provides a method of treating an abnormality in a subject wherein the abnormality is alleviated by the activation of a Y5 receptor which comprises administering to a subject an effective amount of the above-described pharmaceutical composition effective to activate the Y5 receptor in the subject.

[0122] This invention provides a method of treating an abnormality in a subject, wherein the abnormality is alleviated by the inhibition of a Y5 receptor which comprises administering to a subject an effective amount of Y5 receptor antagonist.

[0123] This invention provides a method of treating an abnormality in a subject wherein the abnormality is alleviated by the activation of a Y5 receptor which comprises administering to a subject an effective amount of a Y5 receptor agonist. In a further embodiment, the abnormal condition is anorexia. In a separate embodiment, the abnormal condition is a sexual/reproductive disorder. In another embodiment, the abnormal condition is depression. In another embodiment, the abnormal condition is anxiety.

[0124] In an embodiment, the abnormal condition is gastric ulcer. In a further embodiment, the abnormal condition is memory loss. In a further embodiment, the abnormal condition is migraine. In a further embodiment, the abnormal condition is pain. In a further embodiment, the abnormal condition is epileptic seizure. In a further embodiment, the abnormal condition is hypertension. In a further embodiment, the abnormal condition is cerebral hemorrhage. In a further embodiment, the abnormal condition is shock. In a further embodiment, the abnormal condition is congestive heart failure. In a further embodiment, the abnormal condition is sleep disturbance. In a further embodiment, the abnormal condition is nasal congestion. In a further embodiment, the abnormal condition is diarrhea.

[0125] This invention provides a method of treating obesity in a subject which comprises administering to the subject an effective amount of a Y5 receptor antagonist.

[0126] This invention provides a method of treating anorexia in a subject which comprises administering to the subject an effective amount of a Y5 receptor agonist.

[0127] This invention provides a method of treating bulimia nervosa in a subject which comprises administering to the subject an effective amount of a Y5 receptor antagonist.

[0128] This invention provides a method of inducing a subject to eat which comprises administering to the subject an effective amount of a Y5 receptor agonist. In one embodiment, the subject is a vertebrate. In another embodiment, the subject is a human.

[0129] This invention provides a method of increasing the consumption of a food product by a subject which comprises a composition of the food product and an effective amount of a Y5 receptor agonist. In one embodiment, the subject is a vertebrate. In another embodiment, the subject is a human.

[0130] This invention provides a method of treating abnormalities which are alleviated by reduction of activity of a human Y5 receptor which comprises administering to a subject an amount of the above-described pharmaceutical composition effective to reduce the activity of human Y5 receptor and thereby alleviate abnormalities resulting from overactivity of a human Y5 receptor.

[0131] This invention provides a method of detecting the presence of a human Y5 receptor on the surface of a cell in vitro which comprises contacting the cell with the antibody capable of binding to the human Y5 receptor under conditions permitting binding of the antibody to the receptor, detecting the presence of the antibody bound to the cell, and thereby detecting the presence of a human Y5 receptor on the surface of the cell.

[0132] This invention provides a method of determining the physiological effects of varying levels of activity of a human Y5 receptors which comprises producing a transgenic nonhuman mammal whose levels of human Y5 receptor activity are varied by use of an inducible promoter which regulates human Y5 receptor expression.

[0133] This invention provides a method of determining the physiological effects of varying levels of activity of a human Y5 receptors which comprises producing a panel of transgenic nonhuman mammals each expressing a different amount of human Y5 receptor.

[0134] This invention provides a method for identifying a substance capable of alleviating the abnormalities resulting from overactivity of a human Y5 receptor comprising administering a substance to the above-described transgenic nonhuman mammals, and determining whether the substance alleviates the physical and behavioral abnormalities displayed by the transgenic nonhuman mammal as a result of overactivity of a human Y5 receptor.

[0135] This invention provides a method for identifying a substance capable of alleviating the abnormalities resulting from underactivity of a human Y5 receptor comprising administering the substance to the above-described transgenic nonhuman mammals and determining whether the substance alleviates the physical and' behavioral abnormalities displayed by the transgenic nonhuman mammal as a result of underactivity of a human Y5 receptor.

[0136] This invention provides a method for treating the abnormalities resulting from underactivity of a human Y5 receptor which comprises administering to a subject an amount of the above-described pharmaceutical composition effective to alleviate the abnormalities resulting from underactivity of a human Y5 receptor.

[0137] This invention provides a method for diagnosing a predisposition to a disorder associated with the activity of a specific human Y5 receptor allele which comprises:

a. obtaining DNA of subjects suffering from the disorder; performing a restriction digest of the DNA with a panel of restriction enzymes; c. electrophoretic-ally separating the resulting DNA fragments on a sizing gel;
d. contacting the resulting gel with a nucleic acid probe capable of specifically hybridizing to DNA encoding a human Y5 receptor and labelled with a detectable marker;
e. detecting labelled bands which have hybridized to the DNA encoding a human Y5 receptor 'labelled with a detectable marker to create a unique band pattern specific to the DNA of subjects suffering from the disorder; f. preparing DNA obtained for diagnosis by steps a-e; and g. comparing the unique band pattern specific to the DNA of subjects suffering from the disorder from step e and the DNA obtained for diagnosis from step f to determine whether the patterns are the same or different and to diagnose thereby predisposition to the disorder if the patterns are the same. In an embodiment, a disorder associated with the activity of a specific human Y5 receptor allele is diagnosed.

[0138] This invention provides a method of preparing a purified Y5 receptor which comprises: a. placing the host cell in suitable conditions permitting the production of the Y5 receptor; b. recovering the receptor so produced by the host cells; and c. purifying the receptor so recovered.

[0139] This invention provides a method of preparing the purified human, rat or canine Y5 receptor which comprises: a. constructing a vector adapted for expression in a cell which comprises the regulatory elements necessary for the expression of the nucleic acid in the cell operatively linked to the nucleic acid encoding the human, rat or canine Y5 receptor as to permit expression thereof, wherein the cell is selected from the group consisting of bacterial cells, yeast cells, insect cells and mammalian cells; b. inserting the vector of step a in a suitable host cell; c. incubating the cells of step b under conditions allowing expression of the human, rat or canine Y5 receptor; d. recovering the receptor so produced; and e. purifying the receptor so recovered, thereby preparing a human, rat or canine Y5 receptor.

[0140] This invention will be better understood from the Experimental Details which follow. However, one skilled in the art will readily appreciate that the specific methods and results discussed are merely illustrative of the invention as described more fully in the claims which follow thereafter.

Experimental Details

MATERIALS AND METHODS

cDNA Cloning

[0141] Total RNA was prepared by a modification of the guanidine thiocyanate method (Kingston, 1987), from 5 grams of rat hypothalamus (Rockland, Gilbertsville, PA). Poly A^+RNA was purified with a FastTrack kit (Invitrogen Corp., San Diego, CA). Double stranded (ds) cDNA was synthesized from 7 µg of poly A^+ RNA according to Gubler and Hoffman (Gubler and Hoffman, 1983), except that ligase was omitted in the second strand cDNA synthesis. The resulting DS cDNA was ligated to BstxI/EcoRI adaptors (Invitrogen Corp.), the excess of adaptors was removed by chromatography on Sephacryl 500 HR (Pharmacia-LKB) and the ds-cDNA size selected on a Gen-Pak Fax HPLC column (Millipore Corp., Milford, MA). High molecular weight fractions were ligated in pEXJ.BS (A cDNA cloning expression vector derived from pcEXV-3; Okayama and Berg, 1983; Miller and Germain, 1986) cut by BstxI as described by Aruffo and Seed (Aruffo and Seed, 1987). The ligated DNA was electroporated in E.Coli MC 1061 F^+ (Gene Pulser, Biorad). A total of 3.4 x 10^6 independent clones with an insert mean size of 2.7 kb could be generated. The library was plated on Petri dishes (Ampicillin selection) in pools of 6.9 to 8.2 x 10^3 independent clones. After 18 hours amplification, the bacteria from each pool were scraped, resuspended in 4 mL of LB media and 1.5 mL processed for plasmid purification with a QIAprep-8 plasmid kit (Qiagen Inc, Chatsworth, CA). 1 ml aliquots of each bacterial pool were stored at -85°C in 20% glycerol.

Isolation of a cDNA clone encoding an atypical rat hypothalamic NPY5 receptor

[0142] DNA from pools of ≈ 7500 independent clones was transfected into COS-7 cells by a modification of the DEAE-dextran procedure (Warden and Thorne, 1968). COS-7 cells were grown in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal calf serum, 100 U/ml of penicillin, 100 µg/ml of streptomycin, 2mM L-glutamine (DMEM-C) at 37°c in 5% CO_2. The cells were seeded one day before transfection at a density of 30,000 cells/cm^2 on Lab-Tek chamber slides (1 chamber, Permanox slide from Nunc Inc., Naperville, IL). On the next day, cells were washed twice with PBS, 735 µl of transfection cocktail was added containing 1/10 of the DNA from each pool and DEAE-dextran

(500 µg/ml) in Opti-MEM I serum free media (Gibco®BRL LifeTechnologies Inc. Grand Island, NY). After a 30 min. incubation at 37°C, 3 ml of chloroquine (80 µM in DMEM-C) was added and the cells incubated a further 2.5 hours at 37°C. The media was aspirated from each chamber and 2 ml of 10% DMSO in DMEM-C added. After 2.5 min. incubation at room temperature, the media was aspirated, each chamber washed once with 2 ml PBS, the cells incubated 48 hours in DMEM-C and the binding assay was performed on the slides. After one wash with PBS, positive pools were identified by incubating the cells with 1 nM ($3x10^6$ cpm per slide) of porcine [^{125}I]-PYY (NEN; SA=2200Ci/mmole) in 20 mM Hepes-NaOH pH 7.4, CaCl2 1.26 mM, MgS04 0.81 mM, KH_2PO_4 0.44 mM, KCL 5.4, NaCl 10mM, .1% BSA, 0.1% bacitracin for 1 hour at room temperature. After six washes (three seconds each) in binding buffer without ligand, the monolayers were fixed in 2.5% glutaraldehyde in PBS for five minutes, washed twice for two minutes in PBS, dehydrated in ethanol baths for two minutes each (70, 80, 95, 100%) and air dried. The slides were then dipped in 100% photoemulsion (Kodak type NTB2) at 42°C and exposed in the dark for 48 hours at 4°c in light proof boxes containing drierite. Slides were developed for three minutes in Kodak D19 developer (32 g/l of water), rinsed in water, fixed in Kodak fixer for 5 minutes, rinsed in water, air dried and mounted with Aqua-Mount (Lerner Laboratories, Pittsburgh, PA). Slides were screened at 25x total magnification. A single clone, CG-18, was isolated by SIB selection as described (Mc Cormick, 1987). DS-DNA was sequenced with a Sequenase kit (US Biochemical, Cleveland, OH) according to the manufacturer. Nucleotide and peptide sequence analysis were performed with GCG programs (Genetics Computer group, Madison, WI).

Isolation of the human Y5 homolog

[0143] Using rat oligonucleotide primers in TM 3 (sense primer; position 484-509 in fig. 1A) and in TM 6 (antisense primer; position 1219-1243 in fig. 3A), applicants screened a human hippocampal cDNA library using the polymerase chain reaction. 1 µl (4 x 10^6 bacteria) of each of 450 amplified pools containing each ≈5000 independent clones and representing a total of 2.2 x 10^6 was subjected directly to 40 cycles of PCR and the resulting products analyzed by agarose gel electrophoresis. One of three positive pools was analyzed further and by sib selection a single cDNA clone was isolated and characterized. This cDNA turned out to be full length and in the correct orientation for expression. DS-DNA was sequenced with a sequenase kit (US Biochemical, Cleveland, OH) according to the manufacturer.

Isolation of the canine Y5 homolog

[0144] An alignment of the coding nucleotide sequences of the rat and human Y5 receptors was used to synthesize a pair of PCR primers. A region upstream of TM III which is 100% conserved between rat and human was chosen to synthesize the forward primer CH 156:

5'-TGGATCAGTGGATGTTTGGCAAAG-3' (Seq. I.D. No. 7).

[0145] A region at the carboxy end of the 5-6 loop, immediately upstream of TM6, which is also 100% conserved between rat and human sequences was chosen to synthesize the reverse primer CH153:

5'-GTCTGTAGAAAACACTTCGAGATCTCTT-3' (Seq. I.D. No. 8).

[0146] The primers CH156-CH153 were used to amplify 10 ng of poly (A+) RNA from rat brain that was reverse transcribed using the SSII reverse transcriptase (GibcoBRL, Gaithersburg, MD). PCR was performed on single-stranded cDNA with Taq Polymerase (Perkin Elmer-Roche Molecular Systems, Branchburg, NJ)under the following conditions: 94°C for 1 min, 60°C for 1 min and 72°C for 1 min for 40 cycles. The resulting 798 bp PCR DNA fragment was subcloned in pCR Script (Stratagene, La Jolla, CA) and sequenced using a sequenase kit (USB, Cleveland, OH) and is designated YS-bd-5.

3' and 5' RACE

[0147] The missing 3' and 5' ends of the beagle dog Y5 receptor sequences were isolated by 3' and 5' RACE using a Marathon cDNA amplification kit (Clontech, Palo Alto, CA). From the sequence of the beagle dog PCR DNA fragment described above, the following PCR primers were synthesized:

(3' RACE)

CH 204:

5'-CTTCCAGTGTTTCACAGTCTGGTGG-3' (Seq. I.D. No. 9);

CH 218 (nested primer):

5'-CTGAGCAGCAGGTATTTATGTGTTG-3' (Seq. I.D. No. 10);

(5' RACE)

CH 219:

5'-CTGGATGAAGAATGCTGACTTCTTACAG-3' (Seq. I.D. No. 11);

CH 245 (nested primer):

5'-TTCTTGAGTGGTTCTCTTGAGGAGG-3' (Seq. I.D. No. 12).

[0148] The 3' and 5' RACE reactions were carried out on beagle dog thalamic cDNA according to the kit specifications, with the primers described above. The resulting PCR DNA products (smear of 0.7 to 10 kb) were purified from an agarose gel and reamplified using the nested primers described above. The resulting DNA bands were again purified from an agarose gel and subcloned in pCR Script (Stratagene, La Jolla, CA).

[0149] The nucleotide sequence corresponding to the 3' end of the cDNA was determined and the plasmid designated Y5-bd-8. The nucleotide sequence corresponding to the 5' end will be determined in the near future. Those nucleotide sequences will then be used to synthesize exact primers against the initiation and stop codon regions and those exact primers will then be used to amplify canine thalamic cDNA to generate a PCR product corresponding to the full length coding region of the canine Y5 receptor, using the Expand High Fidelity polymerase (Boehringer Mannheim Corporation, Indianapolis, IN). The resulting PCR DNA product will be subcloned in the expression vector pEXJ and the entire coding region of the canine Y5 nucleotide sequence will be determined using a Sequenase Kit (USB, Cleveland, OH).

Northern Blots

[0150] Human brain multiple tissue northern blots (MTN blots II and III, Clontech, Palo Alto, CA) carrying mRNA purified from various human brain areas was hybridized at high stringency according to the manufacturer specifications.

[0151] The probe was a 0.8 kb DNA PCR fragment corresponding to the TM III - carboxy end of the 5-6 loop in the coding region of the human Y5 receptor subtype.

[0152] A rat multiple tissue northern blot (rat MTN blot, Clontech, Palo Alto, CA) carrying mRNA purified from various rat tissues was hybridized at high stringency according to the manufacturer specifications. The probe was a 0.8 kb DNA PCR fragment corresponding to the TM III - carboxy end of the 5-6 loop in the coding region of the rat Y5 receptor subtype.

Southern Blot

[0153] Southern blots (Geno-Blot, clontech, Palo Alto, CA) containing human or rat genomic DNA cut with five different enzymes (8 µg DNA per lane) was hybridized at high stringency according to the manufacturer specifications. The probe was a .8 kb DNA PCR fragment corresponding to the TM III - carboxy end of the 5-6 loop in the coding region of the human and rat Y5 receptor subtypes.

Production of Recombinant Baculovirus

[0154] A Bam HI site directly 5' to the starting methionine of human Y5 was genetically engineered by replacing the beginning ≈100 base pairs of hY5 (i.e. from the starting methionine to an internal EcoRI site) with two overlapping synthetically-derived oligonucleotides (≈100 bases each), containing a 5' Bam HI site and a 3' EcoRI site. This permitted the isolation of an ≈1.5 kb Bam HI/Hind III fragment containing the coding region of hY5. This fragment was subcloned into pBlueBacIII™ into the Bam HI/Hind III sites found in the polylinker (construct called pBB/hY5). To generate baculovirus, 0.5 µg of viral DNA (BaculoGold™) and 3 µg of pBB/hY5 were cotransfected into 2 x 10^6 Spodoptera frugiperda insect Sf9 cells by calcium phosphate co-precipitation method, as outlined in by Pharmingen (in "Baculovirus Expression Vector System: Procedures and Methods Manual"). The cells were incubated for 5 days at 27°C. The supernatant of the co-transfection plate was collected by centrifugation and the recombinant virus (hY5BB3) was plaque purified. The procedure to infect cells with virus, to prepare stocks of virus and to titer the virus stocks were as described in Pharmingen's manual.

Cell Culture

[0155] COS-7 cells were grown on 150 mm plates in D-MEM with supplements (Dulbecco's Modified Eagle Medium with 10% bovine calf serum, 4 mM glutamine, 100 units/ml penicillin/100 µg/ml streptomycin) at 37°C, 5% CO_2. Stock plates of COS-7 cells were trypsinized and split 1:6 every 3-4 days. Human embryonic kidney 293 cells were grown on 150 mm plates in D-MEM with supplements (minimal essential medium) with Hanks' salts and supplements (Dulbecco's Modified Eagle Medium with 10% bovine calf serum, 4 mM glutamine, 100 units/ml penicillin/100 µg/ml streptomycin) at 37 °C, 5% CO_2. Stock plates of 293 cells were trypsinized and split 1:6 every 3-4 days. Mouse fibroblast LM(tk-) cells were grown on 150 mm plates in D-MEM with supplements (Dulbecco's Modified Eagle Medium with 10% bovine calf serum, 4 mM glutamine, 100 units/mL penicillin/100 µg/mL streptomycin) at 37 °C, 5% CO_2. Stock plates of LM(tk-) cells were trypsinized and split 1:10 every 3-4 days.

[0156] LM(tk-) cells stably transfected with the human Y5 receptor were routinely converted from an adherent monolayer to a viable suspension. Adherent cells were harvested with trypsin at the point of confluence, resuspended in a minimal volume of complete DMEM for a cell count, and further diluted to a concentration of 10^6 cells/ml in suspension media (10% bovine calf serum, 10% 10X Medium 199 (Gibco), 9 mM $NaHCO_3$, 25 mM glucose, 2 mM L-glutamine, 100 units/ml penicillin/100 µg/ml streptomycin, and 0.05% methyl cellulose). The cell suspension was maintained in a shaking incubator at 37 °C, 5% CO2 for 24 hours. Membranes harvested from cells grown in this manner may be stored as large, uniform batches in liquid nitrogen. Alternatively, cells may be returned to adherent cell culture in complete DMEM by distribution into 96-well microtiter plates coated with poly-D-lysine (0.01 mg/ml) followed by incubation at 37 °C, 5% CO_2 for 24 hours. Cells prepared in this manner yielded a robust and reliable NPY-dependent response in cAMP radio-immunoassays as further described hereinbelow.

[0157] Mouse embryonic fibroblast NIH-3T3 cells were grown on 150 mm plates in Dulbecco's Modified Eagle Medium (DMEM) with supplements (10% bovine calf serum, 4 mM glutamine, 100 units/ml penicillin/100 µg/ml streptomycin) at 37 °C, 5% CO2. Stock plates of NIH-3T3 cells were trypsinized and split 1:15 every 3-4 days.

[0158] Sf9 and Sf21 cells were grown in monolayers on 150 mm tissue culture dishes in TMN-FH media supplemented with 10% fetal calf serum, at 27°C, no CO_2. High Five insect cells were grown on 150 mm tissue culture dishes in Ex-Cell 400™ medium supplemented with L-Glutamine, also at 27°C, no CO_2.

Transient Transfection

[0159] All receptor subtypes studied (human and rat Y1, human and rat Y2, human and rat Y4, human and rat Y5) were transiently transfected into COS-7 cells by the DEAE-dextran method, using 1 µg of DNA/10^6 cells (Cullen, 1987). The human Y1 receptor was prepared using known methods (Larhammar, et al., 1992).

Stable Transfection

[0160] Human Y1, human Y2, and rat Y5 receptors were cotransfected with a G-418 resistant gene into the human embryonic kidney 293 cell line by a calcium phosphate transfection method (Cullen, 1987). Stably transfected cells were selected with G-418. Human Y4 and human Y5 receptors were similarly transfected into mouse fibroblast LM (tk-) cells and NIH-3T3 cells.

Expression of other G-protein coupled receptors

[0161] **α_1 Human Adrenergic Receptors:** To determine the binding of compounds to human α_1 receptors, LM(tk-)

cell lines stably transfected with the genes encoding the α_{1a}, α_{1b}, and α_{1d} receptors were used. The nomenclature describing the α_1 receptors was changed recently, such that the receptor formerly designated α_{1a} is now designated α_{1d}, and the receptor formerly designated α_{1c} is now designated α_{1a} (ref). The cell lines expressing these receptors were deposited with the ATCC before the nomenclature change and reflect the subtype desgnations formerly assigned to these receptors. Thus, the cell line expressing the receptor described herein as the α_{1a} receptor was deposited with the ATCC on September 25, 1992, under ATCC Accession No. CRL 11140 with the designation L-α_{1c}. The cell line expressing receptor described herein as the α_{1d} receptor was deposited with the ATCC on September 25, 1992, under ATCC Accession No. CRL 11138 with the designation L-α_{1A}. The cell line expressing the α_{1b} receptor is designated L-α_{1B}, and was deposited on September 25, 1992, under ATCC Accession No. CRL 11139.

[0162] **α_2 Human Adrenergic Receptors:** To determine the binding of compounds to human α_2 receptors, LM(tk-) cell lines stably transfected with the genes encoding the α_{2A}, α_{2B}, and α_{2C} receptors were used. The cell line expressing the α_{2A} receptor is designated L-α_{2A}, and was deposited on November 6, 1992, under ATCC Accession No. CRL 11180. The cell line expressing the α_{2B} receptor is designated L-NGC-α_{2B}, and was deposited on October 25, 1989, under ATCC Accession No. CRL 10275. The cell line expressing the α_{2C} receptor is designated L-α_{2C}, and was deposited on November 6, 1992, under ATCC Accession No. CRL-11181. Cell lysates were prepared as described below (see Radioligand Binding to Membrane Suspensions), and suspended in 25mM glycylglycine buffer (pH 7.6 at room temperature). Equilibrium competition binding assay were performed using [^{3}H]rauwolscine (0.5nM), and nonspecific binding was determined by incubation with 10μM phentolamine. The bound radioligand was separated by filtration through GF/B filters using a cell harvester.

[0163] **Human Histamine H_1 Receptor:** The coding sequence of the human histamine H_1 receptor, homologous to the bovine H_1 receptor, was obtained from a human hippocampal cDNA library, and was cloned into the eukaryotic expression vector pCEXV-3. The plasmid DNA for the H_1 receptor is designated pcEXV-H1, and was deposited on November 6, 1992, under ATCC Accession No. 75346. This construct was transfected into COS-7 cells by the DEAE-dextran method. Cells were harvested after 72 hours and lysed by sonication in 5mM Tris-HCl, 5mM EDTA, pH 7.5. The cell lysates were centrifuged at 1000 rpm for 5 min at 4°C, and the supernatant was centrifuged at 30,000 x g for 20 min. at 4°C. The pellet was suspended in 37.8 mM $NaHPO_4$, 12.2 mM KH_2PO_4, pH 7.5. The binding of the histamine H_1 antagonist [^{3}H]mepyramine (1nM, specific activity: 24.8 Ci/mM) was done in a final volume of 0.25 mL and incubated at room temperature for 60 min. Nonspecific binding was determined in the presence of 10 μM mepyramine. The bound radioligand was separated by filtration through GF/B filters using a cell harvester.

[0164] **Human Histamine H_2 Receptor:** The coding sequence of the human H_2 receptor was obtained from a human placenta genomic library, and cloned into the cloning site of PCEXV-3 eukaryotic expression vector. The plasmid DNA for the H_2 receptor is designated pcEXV-H2, and was deposited on November 6, 1992 under ATCC Accession No. 75345. This construct was transfected into COS-7 cells by the DEAE-dextran method. Cells were harvested after 72 hours and lysed by sonication in 5mM Tris-HCl, 5mM EDTA, pH 7.5. The cell lysates were centrifuged at 1000 rpm for 5 min at 4°C, and the supernatant was centrifuged at 30,000 x g for 20 min at 4 °C. The pellet was suspended in 37.8 mM $NaHPO_4$, 12.2 mM K2PO_4, pH 7.5. The binding of the histamine H_2 antagonist [^{3}H]tiotidine (5nM, specific activity: 70 Ci/mM) was done in a final volume of 0.25 ml and incubated at room temperature for 60 min. Nonspecific binding was determined in the presence of 10 μM histamine. The bound radioligand was separated by filtration through GF/B filters using a cell harvester.

Human Serotonin Receptors:

[0165] $5HT_{1D\alpha}$, $5HT_{1D\beta}$, $5HT_{1E}$, $5HT_{1F}$ Receptors: LM(tk-) clonal cell lines stably transfected with the genes encoding each of these 5HT receptor subtypes were prepared as described above. The cell line for the $5HT_{1D\alpha}$ receptor, designated as Ltk-8-30-84, was deposited on April 17, 1990, and accorded ATCC Accession No. CRL 10421. The cell for the $5HT_{1D\beta}$ receptor, designated as Ltk-11, was deposited on April 17, 1990, and accorded ATCC Accession No. CRL 10422. The cell line for the $5HT_{1E}$ receptor, designated 5 HT_{1E}-7, was deposited on November 6, 1991, and accorded ATCC Accession No. CRL 10913. The cell line for the $5HT_{1F}$ receptor, designated L-5-HT_{1F}, was deposited on December 27, 1991, and accorded ATCC Accession No. ATCC 10957. Membrane preparations comprising these receptors were prepared as described below, and suspended in 50mM Tris-HCl buffer (pH 7.4 at 37°C) containing 10 mM $MgCl_2$, 0.2 mM EDTA, 10μM pargyline, and 0.1% ascorbate. The binding of compounds was determined in competition binding assays by incubation for 30 minutes at 37°C in the presence of 5nM [^{3}H]serotonin. Nonspecific binding was determined in the presence of 10μM serotonin. The bound radioligand was separated by filtration through GF/B filters using a cell harvester.

[0166] **Human $5HT_2$ Receptor:** The coding sequence of the human $5HT_2$ receptor was obtained from a human brain cortex cDNA library, and cloned into the cloning site of pCEXV-3 eukaryotic expression vector. This construct was transfected into COS-7 cells by the DEAE-dextran method. Cells were harvested after 72 hours and lysed by sonication in 5mM Tris-HCl, 5mM EDTA, pH 7.5. This cell line was deposited with the ATCC on October 31, 1989, designated as

L-NGC-5HT$_2$, and was accorded ATCC Accession No. CRL 10287. The cell lysates were centrifuged at 1000 rpm for 5 minutes at 4°C, and the supernatant was centrifuged at 30,000 x g for 20 minutes at 4°C. The pellet was suspended in 50mM Tris-HCl buffer (pH 7.7 at room temperature) containing 10 mM $MgSO_4$, 0.5mM EDTA, and 0.1% ascorbate. The potency of alpha-1 antagonists at 5HT$_2$ receptors was determined in equilibrium competition binding assays using [^{3}H]ketanserin (1nM). Nonspecific binding was defined by the addition of 10µM mianserin. The bound radioligand was separated by filtration through GF/B filters using a cell harvester.

[0167] Human 5-HT$_7$ Receptor: A LM(tk-) clonal cell line stably transfected with the gene encoding the 5HT, receptor subtype was prepared as described above. The cell line for the 5HT$_7$ receptor, designated as L-5HT$_{4B}$, was deposited on October 20, 1992, and accorded ATCC Accession No. CRL 11166.

[0168] Human Dopamine D$_3$ Receptor: The binding of compounds to the human D3 receptor was determined using membrane preparations from COS-7 cells transfected with the gene encoding the human D$_3$ receptor. The human dopamine D$_3$ receptor was prepared according to known methods (Sokoloff, P. et al. Nature, 347, 146, 1990, deposited with the EMBL Genbank as X53944). Cells were harvested after 72 hours and lysed by sonication in 5mM Tris-HCl, 5mM EDTA, pH 7.5. The cell lysates were centrifuged at 1000 rpm for 5 minutes at 4°C, and the supernatant was centrifuged at 30,000 x g for 20 minutes at 4°C. The pellet was suspended in 50 mM Tris-HCl (pH 7.4) containing 1mM EDTA, 5mM KCl, 1.5mM $CaCl_2$, 4mM $MgCl_2$, and 0.1% ascorbic acid. The cell lysates were incubated with [^{3}H]spiperone (2nM), using 10µM (+)Butaclamol to determine nonspecific binding.

Membrane Harvest

[0169] Membranes were harvested from COS-7 cells 48 hours after transient transfection. Adherent cells were washed twice in ice-cold phosphate buffered saline (138 mM NaCl, 8.1 mM Na_2HPO_4, 2.5 mM KCl, 1.2 mM KH_2PO_4, 0.9 mM $CaCl_2$, 0.5 mM $MgCl_2$, pH 7.4) and lysed by sonication in ice-cold sonication buffer (20 mM Tris-HCl, 5 mM EDTA, pH 7.7). Large particles and debris were cleared by low speed centrifugation (200 x g, 5 min, 4 °C). Membranes were collected from the supernatant fraction by centrifugation (32,000 x g, 18 min, 4 °C), washed with ice-cold hypotonic buffer, and collected again by centrifugation (32,000 x g, 18 min, 4 °C). The final membrane pellet was resuspended by sonication into a small volume of ice-cold binding buffer (~1 ml for every 5 plates: 10 mM NaCl, 20 mM HEPES, 0.22 mM KH_2PO_4, 1.26 mM $CaCl_2$, 0.81 mM $MgSO_4$, pH 7.4). Protein concentration was measured by the Bradford method (Bradford, 1976) using Bio-Rad Reagent, with bovine serum albumin as a standard. Membranes were held on ice for up to one hour and used fresh, or flash-frozen and stored in liquid nitrogen.

[0170] Membranes were prepared similarly from 293, LM(tk-), and NIH-3T3 cells. To prepare membranes from baculovirus infected cells, 2 x 10^7 Sf21 cells were grown in 150mm tissue culture dishes and infected with a high-titer stock of hY5BB3. Cells were incubated for 2-4 days at 27°C, no CO_2 before harvesting and membrane preparation as described above.

[0171] Membranes were prepared similarly from dissected rat hypothalamus. Frozen hypothalami were homogenized for 20 seconds in ice-cold sonication buffer with the narrow probe of a Virtishear homogenizer at 1000 rpm (Virtis, Gardiner, NY). Large particles and debris were cleared by centrifugation (200 x g, 5 min, 4 °C) and the supernatant fraction was reserved on ice. Membranes were further extracted from the pellet by repeating the homogenization and centrifugation procedure two more times. The supernatant fractions were pooled and subjected to high speed centrifugation (100,000 x g, 20 min. 4 °C). The final membrane pellet was resuspended by gentle homogenization into a small volume of ice-cold binding buffer (1 mL/ gram wet weight tissue) and held on ice for up to one hour, or flash-frozen and stored in liquid nitrogen.

Radioligand Binding to Membrane Suspensions

[0172] Membrane suspensions were diluted in binding buffer supplemented with 0.1% bovine serum albumin to yield an optimal membrane protein concentration so that ^{125}I-PYY (or alternative radioligand such as ^{125}I-NPY, ^{125}I-PYY$_{3-36}$, or ^{125}I-[Leu^{31}Pro^{34}]PYY) bound by membranes in the assay was less than 10% of ^{125}I-PYY (or alternative radioligand) delivered to the sample (100,000 dpm/sample = 0.08 nM for competition binding assays). ^{125}I-PYY (or alternative radioligand) and peptide competitors were also diluted to desired concentrations in supplemented binding buffer. Individual samples were then prepared in 96-well polypropylene microtiter plates by mixing ^{125}I-PYY (25 µL) (or alternative radioligand), competing peptides or supplemented binding buffer (25 µL), and finally, membrane suspensions (200 µl). Samples were incubated in a 30 °C water bath with constant shaking for 120 min. Incubations were terminated by filtration over Whatman GF/C filters (pre-coated with 1% polyethyleneimine and air-dried before use), followed by washing with 5 mL of ice-cold binding buffer. Filter-trapped membranes were impregnated with MultiLex solid scintillant (Wallac, Turku, Finland) and counted for ^{125}I in a Wallac Beta-Plate Reader. Non-specific binding was defined by 300 nM human NPY for all receptors except the Y4 subtypes; 100 nM human PP was used for the human Y4 and 100 nM rat PP for the rat Y4. Specific binding in time course and competition studies was typically 80%; most nonspecific

binding was associated with the filter. Binding data were analyzed using nonlinear regression and statistical techniques available in the GraphPAD Prism package (San Diego, CA).

Functional Assay: Radioimmunoassay of cAMP

[0173] Stably transfected cells were seeded into 96-well microtiter plates and cultured until confluent. To reduce the potential for receptor desensitization, the serum component of the media was reduced to 1.5% for 4 to 16 hours before the assay. Cells were washed in Hank's buffered saline, or HBS (150 mM NaCl, 20 mM HEPES, 1 mM $CaCl_2$, 5 mM KCl, 1 mM $MgCl_2$, and 10 mM glucose) supplemented with 0.1% bovine serum albumin plus 5 mM theophylline and pre-equilibrated in the same solution for 20 min at 37 °C in 5% CO_2. Cells were then incubated 5 min with 10 µM forskolin and various concentrations of receptor-selective ligands. The assay was terminated by the removal of HBS and acidification of the cells with 100 mM HCl. Intracellular cAMP was extracted and quantified with a modified version of a magnetic bead-based radioimmunoassay (Advanced Magnetics, Cambridge, MA). The final antigen/antibody complex was separated from free ^{125}I-cAMP by vacuum filtration through a PVDF filter in a microtiter plate (Millipore, Bedford, MA). Filters were punched and counted for ^{125}I in a Packard gamma counter. Binding data were analyzed using nonlinear regression and statistical techniques available in the GraphPAD Prism package (San Diego, CA).

Functional Assay: Intracellular calcium mobilization

[0174] The intracellular free calcium concentration was measured by microspectroflourometry using the fluorescent indicator dye Fura-2/AM (ref). Stably transfected cells were seeded onto a 35 mm culture dish containing a glass coverslip insert. Cells were washed with HBS and loaded with 100 µl of Fura-2/AM (10 µM) for 20 to 40 min. After washing with HBS to remove the Fura-2/AM solution, cells were equilibrated in HBS for 10 to 20 min. Cells were then visualized under the 40X objective of a Leitz Fluovert FS microscope and fluorescence emission was determined at 510 nM with excitation wave lengths alternating between 340 nM and 380 nM. Raw fluorescence data were converted to calcium concentrations using standard calcium concentration curves and software analysis techniques.

Tissue preparation for neuroanatomical studies

[0175] Male Sprague-Dawley rats (Charles Rivers) were decapitated and the brains rapidly removed and frozen in isopentane. Coronal sections were cut at 11 µm on a cryostat and thaw-mounted onto poly-L-lysine coated slides and stored at -80° C until use. Prior to hybridization, tissues were fixed in 4% paraformaldehyde, treated with 5 mM dithiothreitol, acetylated in 0.1 M triethanolamine containing 0.25% acetic anhydride, delipidated with chloroform, and dehydrated in graded ethanols.

Probes

[0176] The oligonucleotide probes employed to characterize the distribution of the rat NPY Y5 mRNA were complementary to nucleotides 1121 to 1165 in the 5,6-loop of the rat Y5 mRNA (fig. 3A) 45mer antisense and sense oligonucleotide probes were synthesized on a Millipore Expedite 8909 Nucleic Acid Synthesis System. The probes were then lyophilized, reconstituted in sterile water, and purified on a 12% polyacrylamide denaturing gel. The purified probes were again reconstituted to a concentration of 100 ng/µl, and stored at -20°C.

In Situ Hybridization

[0177] Probes were 3'-end labeled with ^{35}S-dATP (1200 Ci/mmol, New England Nuclear, Boston, MA) to a specific activity of 10^9 dpm/µg using terminal deoxynucleotidyl transferase (Pharmacia). The radiolabeled probes were purified on Biospin 6 chromatography columns (Bio-Rad; Richmond, CA), and diluted in hybridization buffer to a concentration of 1.5×10^4 cpm/µl. The hybridization buffer consisted of 50% formamide, 4X sodium citrate buffer (1X SSC = 0.15 M NaCl and 0.015 M sodium citrate), 1X Denhardt's solution (0.2% polyvinylpyrrolidine, 0.2% Ficoll, 0.2% bovine serum albumin), 50 mM dithiothreitol, 0.5 mg/ml salmon sperm DNA, 0.5 mg/ml yeast tRNA, and 10% dextran sulfate. One hundred µl of the diluted radiolabeled probe was applied to each section, which was then covered with a Parafilm coverslip. Hybridization was carried out overnight in humid chambers at 40 to 55°C. The following day the sections were washed in two changes of 2X SSC for one hour at room temperature, in 2X SSC for 30 min at 50-60°C, and finally in 0.1X SSC for 30 min at room temperature. Tissues were dehydrated in graded ethanols and apposed to Kodak XAR-5 film for 3 days to 3 weeks at -20°C, then dipped in Kodak NTB3 autoradiography emulsion diluted 1:1 with 0.2% glycerol water. After exposure at 4°C for 2 to 8 weeks, the slides were developed in Kodak D-19 developer, fixed, and counterstained with cresyl violet.

Hybridization controls

[0178] Controls for probe/hybridization specificity included hybridization with the radiolabeled sense probe, and the use of transfected cell lines. Briefly, COS-7 cells were transfected (see above) with receptor cDNAs for the rat Y1, Y2 (disclosed in US patent application Serial No. 08/192,288, filed on February 3, 1994), Y4 (disclosed in US patent application Serial No. 08/176,412, filed on December 28 1993), or Y5. As described above, the transfected cells were treated and hybridized with the radiolabeled Y5 antisense and sense oligonucleotide probes, washed, and apposed to film for 1-7 days.

Analysis of hybridization signals

[0179] Sections through the rat brain were analyzed for hybridization signals in the following manner. "Hybridization signal" as used in the present context indicates the relative number of silver grains observed over neurons in a selected area of the rat brain. Two independent observers rated the intensity of the hybridization signal in a given brain area as nonexistent, low, moderate, or high. These were then converted to a subjective numerical scale as 0, +1, +2, or +3 (see Table 10), and mapped on to schematic diagrams of coronal sections through the rat brain (see Fig. 11).

Chemical synthetic methods

Compound 28

2-(Naphthalen-1-ylamino)-3-phenylpropionitrile

[0180] To a solution of 1-naphthalenemethylamine (2.9 g, 20 mmol) and benzylaldehyde (2.0 g, 17 mmol) in 30 ml of $CHCl_3$ and 10 ml of MeOH was added TMSCN (6.6 ml, 51 mmol) and the resulting solution was stirred for 12 h at 25 °C. The reaction mixture was concentrated in vacuo, yielding an oil which was subjected to column chromatography (EtOAc, neat) to provide 3.5 g (74%) of the desired product as a colorless oil. Product was identified by NMR.

2-(Naphthalen-1-yl)-3-phenylpropane-1,2-diamine

[0181] To a solution of the nitrile (0.5 g, 1.8 mmol) in THF was added 6.9 ml of 1N $LiAlH_4$ in THF dropwise and the resulting solution was stirred for 2 h. The reaction was quenched by adding a few pieces of ice into the solution. The reaction mixture was diluted with EtOAc and filtered through pad of Celite. Organic filtrate was concentrated in vacuo to provide a oily residue which was subjected to column chromatography (EtOAc, neat) to provide 0.28 g (57%) of the desired product as a colorless oil. The product was identified by NMR.

***In vivo* Studies in rats**

Food intake in satiated rats

[0182] For these determinations food intake maybe measured in normal satiated rats after intracerebroventricular application (i.c.v.) of NPY in the presence or absence of the test compound. Male Sprague Dawley rats (Ciba-Geigy AG, Sisseln, Switzerland) weighing between 180g and 220 g are used for all experiments. The rats are individually housed in stainless steel cages and maintained on an 11:13 h light-dark cycle (lights off at 18:00 h) at a controlled temperature of 21-23 °C at all times. Water and food (NAFAG lab chow pellets, NAFAG, Gossau, Switzerland) are available ad libidum.

[0183] Rats under pentobarbital anesthesia are stereotaxically implanted with a stainless steel guide cannula targeted at the right lateral ventricle. Stereotaxic coordinates, with the incisor bar set -2.0mm below interaural line, are: -0.8mm anterior and +1.3mm lateral to bregma. The guide cannula is placed on the dura. Injection cannulas extend the guide cannulas -3.8mm ventrally to the skull surface. Animals are allowed at least 4 days of recovery postoperatively before being used in the experiments. Cannula placement is checked postoperatively by testing all rats for their drinking response to a 50 ng intracerebroventricular (i.c.v.) injection of angiotensin II. Only rats which drink at least 2.5 ml of water within 30 min. after angiotensin II injection are used in the feeding studies.

[0184] All injections are made in the morning 2 hours after light onset. Peptides are injected in artificial cerebrospinal fluid (ACSF) in a volume of 5µl. ACSF contains: NaCl 124mM, KCl 3.75 mM, $CaCl_2$ 2.5 mM, $MgSO_4$ 2.0 mM, KH_2PO_4 0.22mM, $NaHCO_3$ 26 mM and glucose 10 mM. porcine-NPY is dissolved in artificial cerebrospinal fluid (ACS). For i. c.v. injection the test compounds are preferably dissolved in DMSO/water (10%, v/v). The vehicle used for intraperitoneal (i.p.) , subcutaneous (s.c.) or oral (p.o.) delivery of compounds is preferably water, physiological saline or DMSO/

water (10% v/v), or cremophor/water (20% v/v), respectively.

[0185] Animals which are treated with both test compounds and p-NPY are treated first with the test compound. Then, 10 min. after i.c.v. application of the test compound or vehicle (control), or 30-60 min after i.p., s.c. and p.o. application of the test compound or vehicle, 300 pmol of NPY is administered by intracerebroventricular (i.c.v.) application.

[0186] Food intake may be measured by placing preweighed pellets into the cages at the time of NPY injection. Pellets are removed from the cage subsequently at each selected time point and replaced with a new set of preweighed pellets. The food intake of animals treated with test compound may be calculated as a percentage of the food intake of control animals, i.e., animals treated with vehicle. Alternatively, food intake for a group of animals subjected to the same experimental condition may be expressed as the mean ± S.E.M. Statistical analysis is performed by analysis of variance using the Student-Newman-Keuls test.

Food intake in food-deprived rats

[0187] Food-deprivation experiments are conducted with male Sprague-Dawley rats weighing between 220 and 250 g. After receipt, the animals are individually housed for the duration of the study and allowed free access to normal food together with tap water. The animals are maintained in a room with a 12 h light/dark cycle (8:00 a.m. to 8:00 p. m. light) at 24 °C and monitored humidity. After placement into individual cages the rats undergo a 4 day equilibration period, during which they are habituated to their new environment and to eating a powdered or pellet diet (NAFAG, Gossau, Switzerland).

[0188] At the end of the equilibration period, food is removed from the animals for 24 hours starting at 8:00 a.m. At the end of the fasting period compound or vehicle may be administered to the animals orally or by injection intraperitoneally or intravenously. After 10 - 60 min. food is returned to the animals and their food intake monitored at various time periods during the following 24 hour period. The food intake of animals treated with test compound may be calculated as a percentage of the food intake of control animals (i.e., animals treated with vehicle). Alternatively, food intake for a group of animals subjected to the same experimental conditions may be expressed as the mean ± S.E.M.

Food intake in obese Zucker rats

[0189] The antiobesity efficacy of the compounds according to the present invention might also be manifested in Zucker obese rats, which are known in the as an animal model of obesity. These studies are conducted with male Zucker fatty rats (fa/fa Harlan CPB, Austerlitz NL) weighing between 480g and 500g. Animals are individually housed in metabolism cages for the duration of the study and allowed free access to normal powdered food and water. The animals are maintained in a room with a 12 h light/dark cycle (light from 8:00 A.M. to 8:00 P.M.) at 24°C and monitored humidity. After placement into the metabolism cages the rats undergo a 6 day equilibration period, during which they are habituated to their new environment and to eating a powdered diet. At the end of the equilibration period, food intake during the light and dark phases is determined. After a 3 day control period, the animals are treated with test compounds or vehicle (preferablywater or physiological saline or DMSO/water (10%,v/v) or cremophor/water (20%,v/v). Food intake is then monitored over the following 3 day period to determine the effect of administration of test compound or vehicle alone. As in the studies described hereinabove, food intake in the presence of drug may be expressed as a percentage of the food intake of animals treated with vehicle.

Materials

[0190] Cell culture media and supplements were from Specialty Media (Lavallette, NJ). Cell culture plates (150 mm and 96-well microtiter) were from Corning (Corning, NY). Sf9, Sf21, and High Five insect cells, as well as the baculovirus transfer plasmid, pBlueBacIII™, were purchased from Invitrogen (San Diego, CA). TMN-FH insect medium complemented with 10% fetal calf serum, and the baculovirus DNA, BaculoGold™, was obtained from Pharmingen (San Diego, CA.). Ex-Cell 400™ medium with L-Glutamine was purchased from JRH Scientific. Polypropylene 96-well microtiter plates were from Co-star (Cambridge, MA). All radioligands were from New England Nuclear (Boston, MA). Commercially available NPY and related peptide analogs were either from Bachem California (Torrance, CA) or Peninsula (Belmont, CA); [D-Trp^{32}]NPY and PP C-terminal fragments were synthesized by custom order from Chiron Mimotopes Peptide Systems (San Diego, CA). Bio-Rad Reagent was from Bio-Rad (Hercules, CA). Bovine serum albumin (ultra-fat free, A-7511) was from Sigma (St. Louis. MO). All other materials were reagent grade.

EXPERIMENTAL RESULTS

cDNA Cloning

[0191] In order to clone a rat hypothalamic "atypical" NPY receptor subtype, applicants used an expression cloning strategy in COS-7 cells (Gearing et al, 1989; Kluxen et al, 1992; Kiefer et al, 1992). This strategy was chosen for its extreme sensitivity since it allows detection of a single "receptor positive" cell by direct microscopic autoradiography. Since the "atypical" receptor has only been described in feeding behavior studies involving injection of NPY and NPY related ligands in rat hypothalamus (see introduction), applicants first examined its binding profile by running competitive displacement studies of ^{125}I-PYY and ^{125}I-PYY$_{3-36}$ on membranes prepared from rat hypothalamus. The competitive displacement data indicate: 1) Human PP is able to displace 20% of the bound ^{125}I-PYY with an IC_{50} of 11 nM (Fig. 1 and Table 2). As can be seen in table 5, this value does not fit with the isolated rat Y1, Y2 and Y4 clones and could therefore correspond to another NPY/PYY receptor subtype. 2) [Leu$_{31}$, Pro$_{34}$] NPY (a Y1 specific ligand) is able to displace with high affinity (IC_{50} of 0.38) 27% of the bound ^{125}I-PYY$_{3-36}$ ligand (a Y2 specific ligand) (Fig. 2 and table 2). These data provide the first evidence based on a binding assay that rat hypothalamic membranes could carry an NPY receptor subtype with a mixed Y1/Y2 pharmacology (referred to as the "atypical" subtype) which fits with the pharmacology defined in feeding behavior studies.

TABLE 2: Pharmacological profile of the rat hypothalamus.

[0192] Binding data reflect competitive displacement of ^{125}I-PYY and ^{125}I-PYY$_{3-36}$ from rat hypothalamic membranes. Peptides were tested at concentrations ranging from 0.001 nM to 100 nM unless noted. The IC_{50} value corresponding to 50% displacement, and the percentage of displacement relative to that produced by 300 nM human NPY, were determined by nonlinear regression analysis. Data shown are representative of at least two independent experiments.

TABLE 2

Peptide	IC_{50} Values, nM (% NPY-produced displacement)	
	^{125}I-PYY	^{125}I-PYY$_{3-36}$
human NPY	0.82 (100%)	1.5 (100%)
human NPY$_{2-36}$	2.3 (100%)	1.2 (100%)
human [Leu^{31},Pro^{34}]NPY	0.21 (44%) 340 (56%)	0.38 (27%) 250 (73%)
human PYY	1.3 (100%)	0.29 (100%)
human PP	11 (20%)	untested

[0193] Based on the above data, a rat hypothalamic cDNA library of 3 x 10^6 independent recombinants with a 2.7 kb average insert size was fractionated into 450 pools of ≈7500 independent clones. All pools were tested in a binding assay with ^{125}I-PYY as previously described (U.S. Serial No. 08/192/288). Seven pools gave rise to positive cells in the screening assay (#'s 81, 92, 147, 246, 254, 290, 312). Since Y1, Y2, Y4 and Y5 receptor subtypes (by PCR or binding analysis) are expressed in rat hypothalamus, applicants analyzed the DNA of positive pools by PCR with rat Y1, Y2 and Y4 specific primers. Pools # 147, 246, 254 and 312 turned out to contain cDNAs encoding a Y1 receptor, pool # 290 turned out tocontain cDNA encoding a Y2 receptor subtype, but pools # 81 and 92 were negative by PCR analysis for Y1, Y2 and Y4 and therefore likely contained a cDNA encoding a new rat hypothalamic NPY receptor (Y5). Pools # 81 and 92 later turned out to contain an identical NPY receptor cDNA. Pool 92 was subjected to sib selection as described in U.S. Serial No. 08/192,288 until a single clone was isolated (designated CG-18).

[0194] The isolated clone carries a 2.8 kb cDNA. This cDNA contains an open reading frame between nucleotides 779 and 2146 that encodes a 456 amino acid protein. The long 5' untranslated region could be involved in the regulation of translation efficiency or mRNA stability. The flanking sequence around the putative initiation codon does not conform to the Kozak consensus sequence for optimal translation initiation (Kozak, 1989, 1991). The hydrophobicity plot displayed seven hydrophobic, putative membrane spanning regions which makes the rat hypothalamic Y5 receptor a member of the G-protein coupled superfamily. The nucleotide and deduced amino acid sequences are shown in Figures 3 and 4, respectively. Like most G-protein coupled receptors, the Y5 receptor contains consensus sequences for N-linked glycosylation in the amino terminus (position 21 and 28) involved in the proper expression of membrane proteins

(Kornfeld and Kornfeld, 1985). The Y5 receptor carries two highly conserved cysteine residues in the first two extracellular loops that are believed to form a disulfide bond stabilizing the functional protein structure (Probst et al, 1992). The Y5 receptor shows 9 potential phosphorylation sites for protein kinase C in positions 204, 217, 254, 273, 285, 301, 328, 336 and 409; and 2 cAMP- and cGMP-dependent protein kinase phosphorylation sites in positions 298 and 370. It should be noted that 8 of these 11 potential phosphorylation sites are located in the third intra-cellular loop, two in the second intra-cellular loop and one in the carboxy terminus of the receptor and could, therefore, play a role in regulating functional characteristics of the Y5 receptor (Probst et al, 1992). In addition, the rat Y5 receptor carries a leucine zipper motif in its first putative transmembrane domain (Landschulz et al, 1988). A tyrosine kinase phosphorylation site is found in the middle of the leucine zipper.

[0195] Localization studies (see below) show that the Y5 mRNA is present in several areas of the rat hippocampus. Assuming a comparable localization in human brain, applicants screened a human hippocampal cDNA library as described in U.S. Serial No. 08/192,288 with rat oligonucleotide primers which were shown to yield a DNA band of the expected size in a PCR reaction run on human hippocampal cDNA (C. Gerald, unpublished results). Using this PCR screening strategy (Gerald et al, 1994, submitted for publication), three positive pools were identified. One of these pools was analyzed further, and an isolated clone was purified by sib selection. The isolated clone (CG-19) turned out to contain a full length cDNA cloned in the correct orientation for functional expression (see below). The human Y5 nucleotide and deduced amino acid sequences are shown in Figures 5 and 6, respectively. When compared to the rat Y5 receptor, the human sequence shows 84.1% nucleotide identity (Fig. 7A to 7E) and 87.2% amino acid identity (Fig. 7F and 7G). The rat protein sequence is one amino acid longer at the very end of both amino and carboxy tails of the receptor when compared to the rat. The human 5-6 loop is one amino acid longer than the rat and shows multiple non conservative substitutions. Even though the 5-6 loops show significant changes between the rat and human homologs, all of the protein motifs found in the rat receptor are present in the human homolog. All putative transmembrane domains and extra cellular loop regions are highly conserved (Fig. 7F and 7G). Therefore, both pharmacological profiles and functional characteristics of the rat and human Y5 receptor subtype homologs may be expected to match closely.

[0196] When the human and rat Y5 receptor sequences were compared to other NPY receptor subtypes or to other human G protein-coupled receptor subtypes, both overall and transmembrane domain identities are very low, showing that the Y5 receptor genes are not closely related to any other previously characterized cDNAs. Even among the human NPY receptor family, Y1, Y2, Y4 and Y5 members show unusually low levels of amino acid identity (Fig. 8A through 8C).

TABLE 3:

Human Y5 transmembrane domains identity with other human NPY receptor subtypes and other human G-protein coupled receptors

Receptor subtype	% TM identity
Y-4	40
Y-2	42
Y-1	42
MUSGIR	32
DroNPY	31
Beta-1	30
Endothelin-1	30
Dopamine D2	29
Adenosine A2b	28
Subst K	28
Alpha-2A	27
5-HT1Dalpha	26
Alpha-1A	26
IL-8	26
5-HT2	25
Subst P	24

Northern blot analysis

[0197] Using the rat Y5 probe, northern hybridizations reveal a strong signal at 2.7 kb and a weak band at 8 kb in rat whole brain. A weak signal is observed at 2.7 kb in testis. No signal was seen in heart, spleen, lung, liver, skeletal muscle and kidney after a three day exposure (Figure 16A). This is in good agreement with the 2.7 kb cDNA that we

isolated by expression cloning from rat hypothalamus and indicates that our cDNA clone is full length. The 8 kb band seen in whole brain probably corresponds to unspliced pre-mRNA.

[0198] With the human Y5 probe, northern hybridizations (Figures 16B and 16C) showed a strong signal at 3.5 kb with a much weaker band at 2.2 and 1.1 kb in caudate nucleus, putamen and cerebral cortex, a medium signal in frontal lobe and amygdala and a weak signal in hippocampus, occipital and temporal lobes, spinal cord, medulla, thalamus, subthalamic nucleus, and substantia nigra. No signal at 3.5 kb was detectable in cerebellum or corpus callosum after a 48 h exposure. It should be noted that Clontech's MTN II and III blots do not carry any mRNA from hypothalamus, periaquiductalgray, superior colliculus and raphe.

[0199] Southern blot analysis on human genomic DNA reveals a unique band pattern in 4 of the 5 restriction digests (Figure 17A). The two bands observed in the PstI digest can be explained by the presence of a PstI site in the coding region of the human Y5 gene. Rat southern blotting analysis showed a unique band pattern in all five restriction digests tested (Figure 17B). These analyses are consistent with the human and rat genomes containing a single copy of the Y5 receptor gene.

Canine Y5 homolog

[0200] The canine nucleotide sequence obtained to date (PCR and 3' RACE products) spans the canine Y5 receptor from the first extracellular loop immediately upstream of TM III into the 3' untranslated region (Figure 14). In the coding region, this nucleotide sequence is highly identical to both the human and the rat sequences (91% and 83.3% respectively). The deduced canine Y5 amino acid sequence is shown in Figure 15. This amino acid sequence is again highly identical to both the human and rat Y5 sequences (94.6% and 89.5% respectively), with most amino acid changes located in the 5-6 loop. Therefore the pharmacological profile of the canine Y5 receptor subtype is expected to closely resemble the human and rat Y5 profiles.

Binding Studies

[0201] The cDNA for the rat hypothalamic Y5 receptor was transiently expressed in COS-7 cells for full pharmacological evaluation. ^{125}I-PYY bound specifically to membranes from COS-7 cells transiently transfected with the rat Y5 receptor construct. The time course of specific binding was measured in the presence of 0.08 nM ^{125}I-PYY at 30 °C (Fig. 9). The association curve was monophasic, with an observed association rate (K_{obs}) of 0.06 min^{-1} and a $t_{1/2}$ of 11 min; equilibrium binding was 99% complete within 71 min and stable for at least 180 min. All subsequent binding assays were carried out for 120 min at 30 °C. The binding of ^{125}I-PYY to transiently expressed rat Y5 receptors was saturable over a radioligand concentration range of 0.4 pM to 2.7 nM. Binding data were fit to a one-site binding model with an apparent K_d of 0.29 nM (pK_d = 9.54 ± 0.13, n = 4). A receptor density of between 5 and 10 pmol/mg membrane protein was measured on membranes which had been frozen and stored in liquid nitrogen (Fig. 10). Membranes from mock-transfected cells, when prepared and analyzed in the same way as those from CG-18-transfected cells, displayed no specific binding of ^{125}I-PYY (data not shown). Applicants conclude that the ^{125}I-PYY binding sites observed under the described conditions were derived from the rat Y5 receptor construct.

[0202] A closely related peptide analog, porcine ^{125}I-[Leu^{31},Pro^{34}]PYY, also bound specifically to membranes from COS-7 cells transiently transfected with rat Y5 receptor cDNA. The time course of specific binding was measured at room temperature in both standard binding buffer ([Na$^+$] = 10 mM) and isotonic binding buffer ([Na$^+$] = 138 mM) using 0.08 nM nM ^{125}I-[Leu^{31},Pro^{34}]PYY nM (Figure 18). The association curve in 10 mM [Na$^+$] was monophasic, with an observed association rate (K_{obs}) of 0.042 min^{-1} and a $t_{1/2}$ of 17 min; equilibrium binding was 99% complete within 110 min and stable for at least 210 min (specific binding was maximal at 480 fmol/mg membrane protein) . The association curve in 138 mM [Na$^+$] was also monophasic with a slightly slower time course: (K_{obs}) of 0.029 min^{-1} and a $t_{1/2}$ of 24 min.; equilibrium binding was 99% complete within 160 min. and stable for at least 210 min. (specific binding was maximal at 330 fmol/mg membrane protein). Note that the specific binding was reduced as [Na$^+$] was increased; a similar phenomenon has been observed for other G protein coupled receptors and may reflect a general property of this receptor family to be modulated by Na$^+$ (Horstman et al., 1990). Saturation binding studies were performed with ^{125}I-[Leu^{31},Pro^{34}]PYY in isotonic buffer at room temperature over a 120 minute period. Specific binding to transiently expressed rat Y5 receptors was saturable over a radioligand concentration range of 0.6 pM to 1.9 nM. Binding data were fit to a one-site binding model with an apparent K_d of 0.072 nM (pKd = 10.14 + 0.07, n = 2). A receptor density of 560 ± 150 pmol/mg on membranes which had been frozen and stored in liquid nitrogen. That ^{125}I-[Leu^{31}, Pro^{34}]PYY can bind to the rat Y5 receptor with high affinity at room temperature in isotonic buffer makes it a potentially useful ligand for characterizing the native Y5 receptor in rat tissues using autoradiographic techniques. Care must be taken, however, to use appropriate masking agents to block potential radiolabeling of other receptors such as Y1 and Y4 receptors (note in Table 5 that rat Y1 and Y4 bind the structural homolog [Pro^{34}]PYY). Previously published reports of ^{125}I-[Leu^{31},Pro^{34}]PYY as a Y1-selective radoligand should be re-evaluated in light of new data obtained with the rat

Y5 receptor (Dumont, et al., 1995).

[0203] The pharmacological profile of the rat Y5 receptor was first studied by using pancreatic polypeptide analogs in membrane binding assays. The rank order of affinity for selected compounds was derived from competitive displacement of ^{125}I-PYY (Fig. 11). The rat Y5 receptor was compared with cloned Y1, Y2, and Y4 receptors from human (Table 4) and rat (Table 5), all expressed transiently in COS-7 cells. One receptor subtype absent from our panel was the Y3, human or rat, as no model suitable for radioligand screening has yet been identified.

TABLE 4: Pharmacological profile of the rat Y5 receptor vs. Y-type receptors cloned from human.

[0204] Binding data reflect competitive displacement of ^{125}I-PYY from membranes of COS-7 cells transiently expressing rat Y5 and human subtype clones. Peptides were tested at concentrations ranging from 0.001 nM to 1000 nM unless noted. IC_{50} values corresponding to 50% displacement were determined by nonlinear regression analysis and converted to K_i values according to the Cheng-Prusoff equation. The data shown are representative of at least two independent experiments.

TABLE 4

Peptide	K_i Values (nM)			
	Rat Y5	Human Y4	Human Y1	Human Y2
rat/human NPY	**0.68**	2.2	0.07	0.74
porcine NPY	**0.66**	1.1	0.05	0.81
human NPY_{2-36}	**0.86**	16	3.9	2.0
porcine NPY_{2-36}	**1.2**	5.6	2.4	1.2
porcine NPY_{13-36}	**73**	38	60	2.5
porcine NPY_{26-36}	**> 1000**	304	> 1000	380
porcine C2-NPY	**470**	120	79	3.5
human [Leu^{31}, Pro ^{34}] NPY	**1.0**	1.1	0.17	> 130
human [D-Trp^{32}]NPY	**53**	> 760	> 1000	> 1000
human NPY free acid	**480**	> 1000	490	> 1000
rat/porcine PYY	**0.64**	0.14	0.35	1.26
human PYY	**0.87**	0.87	0.18	0.36
human PYY_{3-36}	**8.4**	15	41	0.70
human PYY_{13-36}	**190**	46	33	1.5
human [Pro^{34}] PYY	**0.52**	0.12	0.14	> 310
human PP	**5.0**	0.06	77	> 1000
human PP_{2-36}*	**not tested**	0.06	> 40	> 100
human PP_{13-36}*	**not tested**	39	> 100	> 100
rat PP	**180**	0.16	450	> 1000
salmon PP	**0.31**	3.2	0.11	0.17

*Tested only up to 100 nM.

TABLE 5: Pharmacological profile of the rat Y5 receptor vs. Y-type receptors cloned from rat.

[0205] Binding data reflect competitive displacement of ^{125}I-PYY from membranes of COS-7 cells transiently expressing rat Y5 and rat subtype clones. Peptides were tested at concentrations ranging from 0.001 nM to 1000 nM. IC_{50} values corresponding to 50% displacement were determined by nonlinear regression analysis and converted to K_i values according to the Cheng-Prusoff equation. The data shown are representative of at least two independent

experiments. Exception: new peptides (marked with a double asterisk) were tested in one or more independent experiments.

TABLE 5

Peptide	K_i Values (nM)			
	Rat Y5	Rat Y4	Rat Y1	Rat Y2
rat/human NPY	**0.68**	1.7	0.12	1.3
porcine NPY **	**0.66**	1.78	0.06	1.74
frog NPY ** (melanostati n)	**0.71**		0.09	0.65
human NPY_{2-36}	**0.86**	5.0	12	2.6
porcine NPY_{2-36} **	**1.1**	18	1.6	1.6
porcine NPY_{3-36} **	**7.7**	36	91	3.7
porcine NPY_{13-36}	**73**	140	190	31
porcine NPY_{16-36} **	**260**	200	140	35
porcine NPY_{18-36} **	**> 1000**		470	12
porcine NPY_{20-36} **	**> 100**		360	93
porcine NPY_{22-36} **	**> 1000**		> 1000	54
porcine NPY_{26-36} **	**> 1000**		> 1000	> 830
human [Leu^{31}, Pro^{34}] NPY	**1.0**	0.59	0.10	> 1000
porcine ** [Leu^{31}, Pro^{34}] NPY	**1.6**	0.32	0.25	840
human (O-Methyl-Tyr^{21})NPY **	**1.6**			2.3
human NPY free acid **	**> 610**	> 1000	720	> 980
porcine C2-NPY **	**> 260**	22	140	2.6
human NPY_{1-24} amide **	**> 1000**		> 320	> 1000
human [D-Trp^{32}]NPY	**35**	> 630	> 1000	760
rat/porcine PYY	**0.64**	0.58	0.21	0.28
human PYY **	**0.87**		0.12	0.30
human PYY_{3-36} **	**8.4**	15		0.48
human PYY_{13-36} **	**290**		130	14
human [Pro^{34}] PYY	**0.52**	0.19	0.25	> 1000
porcine [Pro^{34}] PYY **	**0.64**	0.24	0.07	> 980
avian PP **	**> 930**	> 81	> 320	> 1000
human PP	**5.0**	0.04	43	> 1000
human PP_{13-36} **	**84**		> 1000	> 650
human PP_{31-36} **	**> 1000**	26	> 10 000	> 10 000
human PP_{31-36} free acid **	**>10,00 0**	> 100		
bovine PP **	**8.4**	0.19	120	> 1000
frog PP (rana temporaria) **	**> 550**	> 1000	720	> 980
rat PP	230	0.19	350	> 1000
salmon PP	**0.33**	3.0	0.30	0.16
PYX-1 **	**920**			

TABLE 5 (continued)

Peptide	K_i Values (nM)			
	Rat Y5	Rat Y4	Rat Y1	Rat Y2
PYX-2 **	**> 1000**			
FLRF-amide **	**5500**		45 000	
FMRF-amide **	**18000**			
W(nor-L)RF-amide **	**8700**			

[0206] The rat Y5 receptor possessed a unique pharmacological profile when compared with human and rat Y-type receptors. It displayed a preference for structural analogs of rat/human NPY (K_i = 0.68 nM) and rat/porcine PYY (K_1 = 0.64 nM) over most PP derivatives. The high affinity for salmon PP (K_i = 0.31 nM) reflects the close similarity between salmon PP and rat NPY, sharing 81% of their amino acid sequence and maintaining identity at key positions: Tyr^1, Gln^{34}, and Tyr^{36}. Both N- and C-terminal peptide domains are apparently important for receptor recognition. The N-terminal tyrosine of NPY or PYY could be deleted without an appreciable loss in binding affinity (K_i = 0.86 nM for rat/human NPY_{2-36}), but further N-terminal deletion was disruptive (K_i = 73 nM for porcine NPY_{13-36}). This pattern places the binding profile of the Y5 receptor somewhere between that of the Y2 receptor (which receptor can withstand extreme N-terminal deletion) and that of the Y1 receptor (which receptor is sensitive to even a single-residue N-terminal deletion). Note that the human Y4 receptor can be described similarly (K_i = 0.06 nM for human PP, 0.06 nM for human PP_{2-36}, and 39 nM for human PP_{13-36}). The Y5 receptor resembled both Y1 and Y4 receptors in its tolerance for ligands containing Pro^{34} (as in human [Leu^{31}, Pro^{34}]NPY, human [Pro^{34}]-PYY, and human PP). Interestingly, the rat Y5 receptor displayed a preference for human PP (K_1 = 5.0 nM) over rat PP (K_1 = 180 nM). This pattern distinguishes the rat Y5 from the rat Y4 receptor, which binds both human and rat PP with K_1 values < 0.2 nM. Hydrolysis of the carboxy terminal amide to free carboxylic acid, as in NPY free acid, was disruptive for binding affinity for the rat Y5 receptor (K_1 = 480 nM). The terminal amide appears to be a common structural requirement for pancreatic polypeptide family/receptor interactions.

[0207] Several peptides shown previously to stimulate feeding behavior in rats bound to the rat Y5 receptor with K_1 ≤ 5.0 nM. These include rat/human NPY (K_1 = 0.68 nM), rat/porcine PYY (K_i = 0.64 nM), rat/human NPY_{2-36} (K_1 = 0.86 nM), rat/human [Leu^{31}, Pro^{34}] NPY (K_i = 1.0 nM), and human PP (K_1 = 5.0 nM). Conversely, peptides which were relatively less effective as orexigenic agents bound weakly to CG-18. These include porcine NPY_{13-36} (K_1 = 73 nM), porcine C2-NPY (K_i = 470 nM) and human NPY free acid (K_i = 480 nM). The rank order of K_i values are in agreement with rank orders of potency and activity for stimulation of feeding behavior when peptides are injected i.c.v. or directly into rat hypothalamus (Clark et al., 1984; Stanley et al., 1985; Kalra et al., 1991; Stanley et al., 1992). The rat Y5 receptor also displayed moderate binding affinity for [$D\text{-}Trp^{32}$]NPY (K_i = 53 nM), the modified peptide reported to regulate NPY-induced feeding by Balasubramaniam and co-workers (1994). It is noteworthy that [$D\text{-}Trp^{32}$]NPY was ≥ 10-fold selective for CG-18 over the other cloned receptors studied, whether human or rat. These data clearly and definitively link the cloned Y5 receptor to the feeding response.

[0208] The cDNA corresponding to the human Y5 homolog isolated from human hippocampus was transiently expressed in COS-7 cells for membrane binding studies. The binding of ^{125}I-PYY to the human Y5 receptor (CG-19) was saturable over a radioligand concentration range of 8 pM to 1.8 nM. Binding data were fit to a one-site binding model with an apparent K_d of 0.10 nM in the first experiment. Repeated testing yielded an apparent K_d of 0.18 nM (pK_d = 9.76 ± 0.11, n = 4). A maximum receptor density of 500 fmol/mg membrane protein was measured on fresh membranes. As determined by using peptide analogs within the pancreatic polypeptide family, the human Y5 pharmacological profile bears a striking resemblance to the rat Y5 receptor (Tables 6 and 7).

TABLE 6: Pharmacological profile of the rat Y5 receptor vs. the human Y5 receptor, as expressed both transiently in COS-7 and stably in LM(tk-) cells.

[0209] Binding data reflect competitive displacement of radioligand (either ^{125}I-PYY or ^{125}I-PYY_{3-36} as indicated) from membranes of COS-7 cells transiently expressing the rat Y5 receptor and its human homolog or from LM(tk-) cells stably expressing the human Y5 receptor. Peptides were tested at concentrations ranging from 0.001 nM to 1000 nM. IC_{50} values corresponding to 50% displacement were determined by nonlinear regression analysis and converted to K_i values according to the Cheng-Prusoff equation. New peptides are marked with a double asterisk.

TABLE 6

Peptide	K_i Values (nM)			
	Rat Y5 (COS-7, ^{125}I-PYY)	Human Y5 (COS-7, ^{125}I-PYY)	Human Y5 (LM (tk-), ^{125}I-PYY)	Human Y5 (LM (tk-), ^{125}I-PYY$_{3-36}$)
rat/human NPY	0.68	0.15	0.89	0.65
porcine NPY **		0.68	1.4	
human NPY$_{2-36}$	0.86	0.33	1.6	0.51
porcine NPY ** 2-36	0.66	0.58	1.2	
porcine NPY$_{13-36}$	73	110		39
porcine NPY$_{16-36}$ **	260	300		180
porcine NPY$_{18-36}$ **	> 1000	> 470		310
porcine NPY$_{22-36}$ **	> 1000	> 1000		
porcine NPY$_{26-36}$ **	> 1000	> 1000		
human [Leu^{31}, Pro ^{34}] NPY	1.0	0.72	3.0	
human [Leu^{31}, Pro ^{34}] NPY **			2.4	1.4
human NPY free acid **	> 610	> 840		
porcine C2-NPY **	260	370	260	220
human [D-Trp^{32}] NPY	35	35	16	10
rat/porci ne PYY	0.64	0.75		
human PYY **	0.87	0.44	1.3	0.43
human PYY$_{3-36}$ **	8.4	17	8.1	1.6
human [Pro^{34}] PYY	0.52	0.34	1.7	1.7
human PP	5.0	1.7	3.0	1.2
human PP$_{2-36}$ **		2.1		
human PP$_{13-36}$ **	290	720		
human PP$_{31-36}$ **	> 10 000	> 10 000		41 000
human [Ile^{31}, Gln ^{34}] PP **		2.0		
bovine PP **	8.4	1.6	7.9	5.0
rat PP	230	630		130
salmon PP	0.33	0.27		0.63

TABLE 7: Pharmacological profile of the human Y5 receptor vs. Y-type receptors cloned from human.

[0210] Binding data reflect competitive displacement of ^{125}I-PYY from membranes of COS-7 cells transiently expressing human Y5 other sub-type clones. Peptides were tested at concentrations ranging from 0.001 nM to 1000 nM unless noted. IC_{50} values corresponding to 50% displacement were determined by nonlinear regression analysis and converted to K_i values according to the Cheng-Prusoff equation. The data shown are representative of at least two independent experiments.

TABLE 7

Peptide	K_i Values (nM)			
	Human Y5	Human Y4	Human Y1	Human Y2
rat/human NPY	**0.46**	2.2	0.07	0.74
porcine NPY	**0.68**	1.1	0.05	0.81
human NPY_{2-36}	**0.75**	16	3.9	2.0
porcine NPY_{2-36}	**0.58**	5.6	2.4	1.2
porcine NPY_{13-36}	**110**	38	60	2.5
porcine NPY_{26-36}	**> 1000**	304	> 1000	380
porcine C2-NPY	**370**	120	79	3.5
human [Leu^{31},Pro^{34}]NPY	**1.6**	1.1	0.17	> 130
human [D-Trp^{32}]NPY	**35**	> 760	> 1000	> 1000
human NPY free acid	**> 840**	> 1000	490	> 1000
rat/porcine PYY	**0.58**	0.14	0.35	1.26
human PYY	**0.44**	0.87	0.18	0.36
human PYY_{3-36}	**17**	15	41	0.70
human PYY_{13-36}	**not tested**	46	33	1.5
human [Pro^{34}] PYY	**0.77**	0.12	0.14	> 310
human PP	**1.4**	0.06	77	> 1000
human PP_{2-36}*	**2.1**	0.06	> 40	> 100
human PP_{13-36}*	**720**	39	> 100	> 100
rat PP	**630**	0.16	450	> 1000
salmon PP	**0.46**	3.2	0.11	0.17

*Tested only up to 100 nM.

Binding Studies of hY5 Expressed in Insect Cells

[0211] Tests were initially performed to optimize expression of hY5 receptor. Infecting Sf9, Sf21, and High Five cells with hY5BB3 virus at a multiplicity of infection (MOI) of 5 and preparing membranes for binding analyses at 45 hours postinfection, we observed B_{max} ranges from 417 to 820 fmoles/mg protein, with the highest expression being hY5BB3 in Sf21 cells. Therefore, our next series of experiments used Sf21 cells. We next examined optimal multiplicity of infection (MOI, the ratio of viral particles to cells) by testing MOI of 1, 2, 5 and 10. The B_{max} values were ≈1.1-1.2 pmoles/mg protein for any of the MOIs, suggesting that increasing the number of viral particles per cell is neither deleterious nor advantageous. Since viral titer calculations are approximate, we used MOI=5 for future experiments. The last parameter we tested was hours postinfection for protein expression, ranging from 45-96 hours postinfection. We found that optimal expression occurred 45-73 hours postinfection. In summary, we have created a hY5 recombinant baculovirus which binds ^{125}I-PYY with a B_{max} of ≈1.2 pmoles/mg protein.

Human Y5 Homolog: Transient Expression in Baculovirus-Infected Sf21 Insect Ovary Cells

[0212] Sf21 cells infected with a human Y5 baculovirus construct were harvested as membrane homogenates and screened for specific binding of ^{125}I-PYY using 0.08 nM radioligand. Specific binding was greatest (500 fmol/mg membrane protein) for sample D-2/[4], derived from Sf-21 cells. No specific binding was observed after infection with the baculovirus plasmid alone (data not shown). If we make the assumption that the binding affinity of porcine ^{125}I-PYY for the human Y5 receptor is the same whether the expression system is COS-7 or baculovirus/Sf-21 (0.18 nM), the specific binding in sample D-2/[4] predicts an apparent B_{max} of 1600 fmol/mg membrane protein. The Y5 receptor yield in the baculovirus/Sf21 expression system is therefore as good or better than that in COS-7. We conclude that the

baculovirus offers an alternative transfection technique amenable to large batch production of the human Y5 receptor.

Stable Expression Systems for Y5 Receptors: Characterization in Binding Assays

[0213] The cDNA for the rat Y5 receptor was stably transfected into 293 cells which were pre-screened for the absence of specific ^{125}I-PYY binding (data not shown). After co-transfection with the rat Y5 cDNA plus a G-418-resistance gene and selection with G-418, surviving colonies were screened as membrane homogenates for specific binding of ^{125}I-PYY using 0.08 nM radioligand. A selected clone (293 clone # 12) bound 65 fmol ^{125}I-PYY /mg membrane protein and was isolated for further study in functional assays.

[0214] The cDNA for the human Y5 receptor was stably transfected into both NIH-3T3 and LM(tk-) cells, each of which were pre-screened for the absence of specific ^{125}I-PYY binding (data not shown). After co-transfection with the human Y5 cDNA plus a G-418-resistance gene and selection with G-418, surviving colonies were screened as membrane homogenates for specific binding of ^{125}I-PYY using 0.08 nM radioligand. NIH-3T3 clone #8 bound 46 fmol 125I-PYY/mg membrane protein and LM(tk-) clone #7 bound 32 fmol ^{125}I-PYY/mg membrane protein. These two clones were isolated for further characterization in binding and cAMP functional assays. A third clone which bound 25 fmol/mg membrane protein, LM(tk-) #3, was evaluated in calcium mobilization assays.

[0215] The human Y5 stably expressed in NIH-3T3 cells (clone #8) was further characterized in saturation binding assays using ^{125}I-PYY. The binding was saturable over a concentration range of 0.4 pM to 1.9 nM. Binding data were fit to a one-site binding model with an apparent K_d of 0.30 nM (pK_d = 9.53, n = 1) and an apparent B_{max} of 2100 fmol/mg membrane protein using fresh membranes.

[0216] The human Y5 stably expressed in LM(tk-) cells (clone #7) was further characterized in saturation binding assays using ^{125}I-PYY, ^{125}I-PYY$_{3-36}$, and ^{125}I-NPY. ^{125}I-PYY binding was saturable according to a 1-site model over a concentration range of 0.4 pM to 1.9 nM, with an apparent K_d of 0.47 nM ($pK_d = 9.32 \pm 0.07$, n =5) and an apparent B_{max} of up to 8 pmol/mg membrane protein when membranes had been frozen and stored in liquid nitrogen. Peptide K_i values derived from ^{125}I-PYY binding to human Y5 receptors from LM(tk-) were comparable to those derived from the previously described human and rat Y5 expression systems (Table 6). ^{125}I-PYY$_{3-36}$ binding to the human Y5 in LM (tk-) cells was also saturable according to a 1-site model over a concentration range of 0.5 pM to 2.09 nM, with an apparent K_d of 0.40 nM (pK_d = 9.40, n = 1) and an apparent B_{max} of 490 fmol/mg membrane protein when membranes had been frozen and stored in liquid nitrogen. Peptide ligands appeared to bind with comparable affinity to human Y5 receptors in LM(tk-) cells whether the radioligand used was ^{125}I-PYY or ^{125}I-PYY$_{3-36}$ (Table 6). Finally, ^{125}I-NPY binding to the human Y5 in LM(tk-) cells was saturable according to a 1-site model over a concentration range of 0.4 pM to 1.19 nM, with an apparent K_d of 0.28 and an apparent B_{max} of 360 fmol/mg membrane protein when membranes had been frozen and stored in liquid nitrogen.

[0217] Considering the saturation binding studies for the human and rat Y5 receptor homologs as a whole, the data provide evidence that the Y5 receptor is a target for multiple radioiodinated peptide analogs in the pancreatic polypeptide family, including ^{125}I-PYY, ^{125}I-NPY, ^{125}I-PYY$_{3-36}$, and ^{125}I-[Leu^{31},Pro^{34}]PYY. The so-called Y1 and Y2-selective radioligands such as ^{125}I-[Leu^{31},Pro^{34}]PYY and ^{125}I-PYY$_{3-36}$, respectively (Dumont, et al., 1995) should be used with caution when probing native tissues for Y-type receptor expression.

Receptor/G protein Interactions: Effects of Guanine Nucleotides

[0218] For a given G protein-coupled receptor, a portion of the receptor population can typically be characterized in the high affinity ligand binding site using discriminating agonists. The binding of GTP or a non-hydrolyzable analog to the G protein causes a conformational change in the receptor which favors a low affinity ligand binding state. We investigated whether the non-hydrolyzable GTP analog, Gpp(NH)p, would alter the binding of ^{125}I-PYY to Y5 in COS-7 and LM(tk-) cells (Fig 19). ^{125}I-PYY binding to both human and rat Y5 receptors in COS-7 cells was relatively insensitive to increasing concentrations of Gpp(NH)p ranging from 1 nM to 100 μM. The human Y5 receptor in LM(tk-) cells, however, displayed a concentration dependent decrease in radioligand binding (-85 fmol/mg membrane protein over the entire concentration range). The difference between the receptor preparations could be explained by several factors, including 1) the types of G proteins available in the host cell for supporting a high affinity receptor-agonist complex, 2) the level of receptor reserve in the host cell, and 3) the efficiency of receptor/G protein coupling, and 4) the intrinsic ability of the agonist (in this case, ^{125}I-PYY) to distinguish between multiple conformations of the receptor.

Functional Assay

[0219] Activation of all Y-type receptors described thus far is thought to involve coupling to pertussis toxin-sensitive G-proteins which are inhibitory for adenylate cyclase activity (G_i or G_o) (Wahlestedt and Reis, 1993). That the atypical Y1 receptor is linked to cyclase inhibition was prompted by the observation that pertussis toxin inhibited NPY-induced

feeding *in vivo* (Chance et al., 1989); a more definitive analysis was impossible in the absence of the isolated receptor. Based on these prior observations, applicants investigated the ability of NPY to inhibit forskolin-stimulated cAMP accumulation in human embryonic kidney 293 cells stably transfected with rat Y5 receptors. Incubation of intact cells with 10 μM forskolin produced a 10-fold increase in CAMP accumulation over a 5 minute period, as determined by radioimmunoassay. Simultaneous incubation with rat/human NPY decreased the forskolin-stimulated cAMP accumulation by 67% in stably transfected cells (Fig. 12), but not in untransfected cells (data not shown). Applicants conclude that the rat Y5 receptor activation results in decreased cAMP accumulation, very likely through inhibition of adenylate cyclase activity. This result is consistent with the proposed signalling pathway for all Y-type receptors and for the atypical Y1 receptor in particular.

[0220] Peptides selected for their ability to stimulate feeding behavior in rats were able to activate the rat Y5 receptor with EC_{50} < 10 nM (Kalra et al., 1991; Stanley et al., 1992; Balasubramaniam et al., 1994). These include rat/human NPY (EC_{50} = 1.8 nM), rat/human NPY_{2-36} (EC_{50} = 2.0 nM), rat/human [Leu^{31}, Pro^{34}]NPY (EC_{50} = 0.6 nM), rat/porcine PYY (EC_{50} = 4.0 nM), and rat/human [$D\text{-}Trp^{32}$]NPY (EC_{50} = 7.5 nM) (Table 8). K_i values derived from rat Y5-dependent binding of ^{125}I-PYY and peptide ligands (Table 5) were in close range of EC_{50} values derived from rat Y5-dependent regulation of cAMP accumulation (Table 8). The maximal suppression of cAMP produced by all peptides in Table 6 was between 84% and 120% of that produced by human NPY, except in the case of FLRFamide (42%). Of particular interest is the Y5-selective peptide [$D\text{-}Trp^{32}$]NPY. This is a peptide which was shown to stimulate food intake when injected into rat hypothalamus, and which also attenuated NPY-induced feeding in the same paradigm (Balasubramaniam, 1994). Applicants observed that [$D\text{-}Trp^{32}$]NPY bound weakly to other Y-type clones with K_i > 500 nM (Tables 4 and 5) and displayed no activity in functional assays (Table 10). In striking contrast, [$D\text{-}Trp^{32}$]NPY bound to the rat Y5 receptor with a K_i = 53 nM and was fully able to mimic the inhibitory effect of NPY on forskolin-stimulated CAMP accumulation with an EC_{50} of 25nm and an E_{max} = 72%. That [$D\text{-}Trp^{32}$]NPY was able to selectively activate the Y5 receptor while having no detectable activity at the other subtype clones strongly suggests that Y5 receptor activation is responsible for the stimulatory effect of [$D\text{-}Trp^{32}$]NPY on feeding behavior *in vivo*.

TABLE 8: Functional activation of the rat Y5 receptor.

[0221] Functional data were derived from radioimmunoassay of CAMP accumulation in stably transfected 293 cells stimulated with 10 μM forskolin. Peptides were tested for agonist activity at concentrations ranging from 0.03 pM to 0.3 μM. The maximum inhibition of CAMP accumulation (E_{max}) and the concentration producing a half-maximal effect (EC_{50}) were determined by nonlinear regression analysis according to a 4 parameter logistic equation. New peptides are marked with a double asterisk.

TABLE 8

Peptide	E_{max}	EC_{50} (nM)
rat/human NPY	67 %	1.8
porcine NPY **		0.79
rat/human NPY_{2-36}	84 %	2.0
porcine NPY_{2-36} **		1.2
porcine NPY_{13-36} **		21
rat/human [Leu^{31}, Pro^{34}] NPY	70 %	0.6
porcine [Leu^{31}, Pro^{34}] NPY **		1.1
porcine C2-NPY **		240
rat/human [$D\text{-}Trp^{32}$] NPY	72 %	9.5
rat/porcine PYY	86 %	4.0
human PYY **		1.5
human PYY_{3-36} **		4.9
human [Pro^{34}] PYY **		1.8
human PP **		1.4
bovine PP **		5.7

TABLE 8 (continued)

Peptide	E_{max}	EC_{50} (nM)
salmon PP **		0.92
rat PP **		130
PYX-1 **		> 300
PYX-2 **		> 300
FLRFamide **		13 000

[0222] The ability of the human Y5 receptor to inhibit cAMP accumulation was evaluated in NIH-3T3 and LM(tk-) cells, neither of which display an NPY-dependent regulation of [cAMP] without the Y5 construct. Intact cells stably transfected with the human Y5 receptor were analyzed as described above for the rat Y5 cAMP assay. Incubation of stably transfected NIH-3T3 cells with 10 uM forskolin generated an average 21-fold increase in [cAMP] (n = 2). Simultaneous incubation with human NPY decreased the forskolin-stimulated [cAMP] with an E_{max} of 42% and an EC_{50} of 8.5 nM (Fig 20). The technique of suspending and then replating the Y5-transfected LM(tk-) cells was correlated with a robust and reliable cellular response to NPY-like peptides and was therefore incorporated into the standard methodology for the functional evaluation of the human Y5 in LM(tk-). Incubation of stably transfected LM(tk-) cells prepared in this manner produced an average 7.4-fold increase in [cAMP] (n = 87). Simultaneous incubation with human NPY decreased the forskolin-stimulated [cAMP] with an E_{max} of 72% and with an EC_{50} of 2.4 nM (Fig 21). The human Y5 receptor supported a cellular response to NPY-like peptides in a rank order similar to that described for the rat Y5 receptor (Table 8, 9). As the rat Y5 receptor is clearly linked by D-Trp32-NPY and other pharmacological tools to the NPY-dependent regulation of feeding behavior, the human Y5 receptor is predicted to function in a similar fashion. Both the human and receptor homologs represent useful models for the screening of compounds intended to modulate feeding behavior by interfering with NPY-dependent pathways.

TABLE 9: Functional activation of the human Y5 receptor in a cAMP radioimmunoassay.

[0223] Functional data were derived from radioimmunoassay of cAMP accumulation in stably transfected LM(tk-) cells stimulated with 10 µM forskolin. Peptides were tested for agonist activity at concentrations ranging from 0.03 pM to 0.3 µM. The maximum inhibition of cAMP accumulation (E_{max}) and the concentration producing a half-maximal effect (EC_{50}) were determined by nonlinear regression analysis according to a 4 parameter logistic equation.

TABLE 9

Peptide	% inhibition relative to human NPY	EC_{50} (nM)
rat/human NPY	100%	2.7
porcine NPY	107%	0.99
rat/human NPY_{2-36}	116%	2.6
porcine NPY_{2-36}	85%	0.71
porcine NPY_{13-36}		49
rat/human [Leu^{31}, Pro^{34}]NPY		3.0
porcine [Leu^{31},Pro^{34}]NPY		1.3
rat/human [D-Trp^{32}]NPY	108%	26
rat/porcine PYY	109%	3.6
human PYY	111%	4.9
human PYY_{3-36}		18
human [Pro^{34}]PYY	108%	2.5
human PP	96%	14
human PP_{2-36}		2.0

TABLE 9 (continued)

Peptide	% inhibition relative to human NPY	EC_{50} (nM)
human [Ile^{31},Gln^{34}]PP		5.6
bovine PP		4.0
salmon PP	96%	4.5

TABLE 10: Binding and functional characterization of [D-Trp^{32}]NPY.

[0224] Binding data were generated as described in Tables 4 and 5. Functional data were derived from radioimmunoassay of cAMP accumulation in stably transfected cells stimulated with 10 μM forskolin. [D-Trp^{32}]NPY was tested for agonist activity at concentrations ranging from 0.03 pM to 0.3 μM. Alternatively, [D-Trp^{32}]NPY was included as a single spike (0.3 μM) in the human PYY concentration curve for human Y1 and human Y2 receptors, or in the human PP concentration curve for human Y4 receptors, and antagonist activity was detected by the presence of a rightward shift (from EC_{50} to EC_{50}'). K_b values were calculated according to the equation: K_b = [[D-Trp^{32}]NPY/((EC_{50}/EC_{50}')-1). The data shown are representative of at least two independent experiments.

TABLE 10

Recept or Subtyp e	Species	Binding	Function		
		K_i (nM)	EC_{50} (nM)	K_b (nM)	Activity
Y1	Human	> 1000			None detected
Y2	Human	> 1000			None detected
Y4	Human	> 1000			None detected
Y5	Human	18	**26**		Not Determined
Y1	Rat	> 1000			Not Determined
Y2	Rat	>1000			Not Determined
Y4	Rat	> 1000			Not Determined
Y5	Rat	53	**9.50**		Agonist

Functional Assay: Intracellular Calcium Mobilization

[0225] The intracellular free calcium concentration was increased in LM(tk-) cells stably transfected with the human Y5 receptor within 30 seconds of incubation with 100 nM human NPY (Δ Ca^{2+} = 34, Fig 21D). Untransfected LM(tk-) cells did not respond to human NPY (data not shown). The calcium mobilization provides a second pathway through which Y5 receptor activation can be measured. These data also serve to link with the Y5 receptor with other cloned human Y-type receptors, all of which have been demonstrated to mobilize intracellular calcium in various expression systems (Fig 21).

Localization Studies

[0226] The mRNA for the NPY Y5 receptor was widely distributed in rat brain, and appeared to be moderately abundant (Table 11 and Fig. 13). The midline thalamus contained many neurons with silver grains over them, particularly the paraventricular thalamic nucleus, the rhomboid nucleus, and the nucleus reunions. In addition, moderately intense hybridization signals were observed over neurons in both the centromedial and anterodorsal thalamic nuclei. In the hypothalamus, a moderate level of hybridization signal was seen over scattered neurons in the lateral hypothalamus, paraventricular, supraoptic, arcuate, and dorsomedial nuclei. In both the medial preoptic nucleus and suprachiasmatic nucleus, weak or moderate accumulations of silver grains were present. In the suprachiasmatic nucleus, hybridization signal was. restricted mainly to the ventrolateral subdivision. In the paraventricular hypothalamus, positive neurons were observed primarily in the medial parvicellular subdivision.

TABLE 11:

Distribution of NPY Y5 mRNA in the Rat CNS	
REGION	**Y5 mRNA**
Cerebral cortex	+1
Thalamus	
paraventricular n.	+3
rhomboid n.	+3
reunions n.	+3
anterodorsal n.	+2
Hypothalamus	
paraventricular n.	+2
lateral hypoth. area	+2 /+3
supraoptic n.	+1
medial preoptic n.	+2
suprachiasmatic n.	+1/+2
arcuate n.	+2
Hippocampus	
dentate gyrus	+1
polymorph dentate gyrus	+2
CA1	0
CA3	+1
Amygdala	
central amygd. n., medial	+2
anterior cortical amygd. n.	+2
Olivary pretectal n.	+3
Anterior pretectal n.	+3
Substantia nigra, pars compacta	+2
Superior colliculus	+2
Central gray	+2
Rostral linear raphe	+3
Dorsal raphe	+1
Inferior colliculus	+1
Medial vestibular n.	+2/+3
Parvicellular ret. n.,alpha	+2
Gigantocellular reticular n., alpha	+2
Pontine nuclei	+1/+2

[0227] Moderate hybridization signals were found over most of the neurons in the polymorphic region of the dentate gyrus in the hippocampus, while lower levels were seen over scattered neurons in the CA3 region. In the amygdala, the central nucleus and the anterior cortical nucleus contained neurons with moderate levels of hybridization signal. In the mesencephalon, hybridization signals were observed over a number of areas. The most intense signals were

found over neurons in the anterior and olivary pretectal nuclei, periaquaductal gray, and over the rostral linear raphe. Moderate hybridization signals were observed over neurons in the internal gray layer of the superior colliculus, the substantia nigra, pars compacta, the dorsal raphe, and the pontine nuclei. Most of the neurons in the inferior colliculus exhibited a low level of signal. In the medulla and pons, few areas exhibited substantial hybridization signals. The medial vestibular nucleus was moderately labeled, as was the parvicellular reticular nucleus, pars alpha, and the gigantocellular reticular nucleus.

[0228] Little or no hybridization signal was observed on sections hybridized with the radiolabeled sense oligonucleotide probe. More importantly, in the transfected COS-7 cells, the antisense probe hybridized only to the cells transfected with the rat Y5 cDNA (Table 12). These results indicate that the probe used to characterize the distribution of Y5 mRNA in rat brain is specific for this mRNA, and does not cross-hybridize to any of the other known NPY receptor mRNAs.

TABLE 12:

Hybridization of antisense oligonucleotide probes to transfected COS-7 cells.					
Hybridization was performed as described in Methods. The NPY Y5 probe hybridizes only to the cells transfected with the Y5 cDNA. ND=not done.					
Cells	Mock	rY1	rY2	rY4	rY5
Oligo					
rY1	-	+	-	ND	ND
rY2	-	-	+	-	-
rY4	-	-	-	+	-
rY5	-	-	-	-	+

In vivo studies with Y5-selective compounds

[0229] The results reported above strongly support a role for the Y5 receptor in regulating feeding behavior. Accordingly, applicants have synthesized and evaluated the binding and functional properties of several compounds at the cloned human Y1, human Y2, human Y4, and human Y5 receptors. As shown below in Table 13, applicants have discovered several compounds which not only bind selectively to the human Y5 receptor but also act as Y5 receptor antagonists, as measured by their ability to block NPY-induced inhibition of cAMP accumulation in forskolin-stimulated LM(tk-) cells stably transfected with the cloned human Y5 receptor. An example of such a compound is shown in Figure 22. Preliminary experiments indicate that compound 28 is a Y5 receptor antagonist.

Table 13: Evaluation of human Y5 receptor antagonists

[0230] The ability of the compounds to antagonize the Y-type receptors is reported as the K_b. The K_b is derived from p the EC_{50}, or concentration of half-maximal effect, in the presence (EC_{50}) or absence (EC_{50}') of compound, according to the equation: $K_b = [NPY]/((EC_{50}/EC_{50}')-1)$. Results shown are representative of at least three indepenent experiments.

N.D. = Not determined.

Table 13

	Binding Affinity (K_i (nM) vs. ^{125}I-PYY)				
Compound	Human Receptor				K_b (nM)
-	Y1	Y2	Y4	Y5	-
1	1660	1920	4540	38.9	183
2	1806	386	1280	17.8	9.6
5	3860	249	2290	1.27	2.1
6	4360	4610	32,900	47.5	93
7	2170	2870	7050	42.0	105

Table 13 (continued)

	Binding Affinity (K_i (nM) vs. ^{125}I-PYY)				
Compound	Human Receptor				K_b (nM)
9	3240	>100,000	3720	108	479
10	1070	>100,000	5830	40.7	2.8
11	1180	>100,000	7130	9.66	1.5
17	5550	1000	8020	14	6.0
19	3550	955	11700	11	23
20	16000	7760	20400	8.3	26
21	13000	1610	18500	9.8	16
22	17200	7570	27500	11	3.0
23	14500	617	21500	26	38
25	3240	851	13100	17	311
26	23700	58200	19300	14	50
27	48700	5280	63100	28	49
28	>100,000	>75,000	>100,000	19,000	N.D.

[0231] These compounds were further tested using in vivo animal models of feeding behavior. Since NPY is the strongest known stimulant of feeding behavior, experiments were performed with several compounds to evaluate the effect of the compounds described above on NPY-induced feeding behavior in satiated rats.

[0232] First, 300 pmole of porcine NPY in vehicle (A.C.S.F.) was administered by intracerebroventricular (i.c.v.) injection, along with i.p. administration of compound vehicle (10% DMSO/water), and the food intake of NPY-stimulated animals was compared to food intake in animals treated with the vehicles. The 300 pmole injection of NPY was found to significantly induce food intake ($p < 0.05$; Student-Newman-Keuls).

[0233] Using the 300 pmole dose of NPY found to be effective to stimulate feeding, other animals were treated with the compounds by intraperitoneal (i.p.) administration, followed 30-60 minutes later by i.c.v. NPY administration, and measurement of subsequent food intake. As shown in Table 14, NPY-induced food intake was significantly reduced in animals first treated with the compounds ($p < 0.05$; Student-Newman-Keuls). These experiments demonstrate that NPY-induced food intake is significantly reduced by administration to animals of a compound which is a Y5-selective antagonist.

Table 14. NPY-induced cumulative food intake in rats treated with either the i.c.v. and i.p. vehicles (control), 300 pmole NPY alone (NPY), or in rats treated first with compound and then NPY (NPY + compound). Food intake was measured 4 hours after stimulation with NPY. Food intake is reported as the mean ± S.E.M. intake for a group of animals.

[0234]

Table 14

	Food intake (g) mean ± S.E.M.			
Compound	1	5	17	19
Compound Dose (mg/kg i.p.)	10	10	10	30
control (vehicles only)	3.7 ± 0.6	2.4 ± 0.5	2.4 ± 0.7	2.9 ± 0.8
NPY	7.4 ± 0.5	6.8 ± 1.0	5.8 ± 0.5	4.9 ± 0.4
NPY + compound	4.6 ± 0.6	4.1 ± 0.4	3.8 ± 0.4	1.5 ± 0.6

[0235] Since food deprivation induces an increase in the hypothalamic NPY levels, it has been postulated that food

intake following a period of food deprivation is NPY-mediated. Therefore, the Y5 antagonists of Table 13 were administered to conscious rats following a 24h food deprivation. Each of the human Y5 receptor antagonists shown in Table 13 was able to significantly reduce NPY-induced food intake in the animals, as shown below in Table 15. The food intake intake of animals treated with test compound is reported as a percentage of the food intake measured for control animals (treated with vehicle), i.e., 25% means the animals treated with the compound consumed only 25% as much food as the control animals. Measurements were performed two hours after administration of the test compound.

Table 15

Two-hour food intake of NPY-stimulated rats.			
Food intake is expressed as the percentage of intake compared to control rats.			
Compound	Mean (%)	Compound	Mean (%)
1	34	**19**	36
2	42	**20**	35
5	87	**21**	80
6	38	**22**	55
7	47	**23**	58
9	40	**25**	32
10	74	**26**	73
11	15	**27**	84
17	27	**28**	N.D.

[0236] These experiments indicate that the compounds of the present invention inhibit food intake in rats, especially when administered in a range of about 0.01 to about 100 mg/kg rat, by either oral, intraperitoneal or intravenous administration. The animals appeared normal during these experiments, and no ill effects on the animals were observed after the termination of the feeding experiments.

[0237] The binding properties of the compounds were also evaluated with respect to other cloned human G-protein coupled receptors. As shown in Table 16, below, the Y5-selective compounds described hereinabove exhibited lower affinity for receptors other than the Y-type receptors.

Table 16 Cross-reactivity of compounds at other cloned human receptors

Compound	Receptor (pKi)								
	α_{1d}	α_{1b}	α_{1a}	α_{2a}	α_{2b}	α_{2c}	H1	H2	D3
1	6.25	6.23	6.15	6.28	6.01	6.34	5.59	6.32	5.69
2	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
5	7.24	7.36	7.63	7.39	7.29	7.63	6.65	6.68	7.24
6	5.68	5.73	6.54	7.14	5.79	6.35	N.D.	N.D.	N.D.
7	6.46	6.08	6.06	7.16	6.09	6.85	N.D.	N.D.	N.D.
9	6.45	6.26	6.57	7.04	5.00	6.81	N.D.	N.D.	N.D.
10	6.12	5.82	6.27	8.94	5.62	6.18	N.D.	N.D	N.D.
11	7.03	5.6	6.05	7.38	5.60	6.00	N.D.	N.D.	N.D.
17	6.68	7.17	7.08	6.52	6.51	7.07	6.33	5.92	6.61
19	6.90	7.35	7.47	6.74	6.58	7.07	7.04	6.29	6.69
20	7.01	7.22	7.72	7.31	6.96	7.39	6.73	5.85	6.35
21	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
22	6.80	6.98	7.34	7.05	6.43	7.15	6.22	5.72	6.29
23	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D	N.D.
25	6.66	6.67	7.07	6.21	5.95	6.79	6.43	6.43	5.93

Table 16 continued

Compound	Receptor (pKi)									
26	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
27	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.

Table 16 continued

Compound	Receptor (pKi)							
	$5HT_{1a}$	$5HT_2$	$5HT_7$	$5HT_{1F}$	$5HT_{1E}$	$5HT_{1D\beta}$	$5HT_{1D\alpha}$
1	4.51	6.34	6.20	5.30	5.30	5.30	5.42
2	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D
5	6.33	6.41	6.00	5.30	5.30	5.55	5.37
6	N.D.	N.D.	6.00	5.30	5.30	5.30	5.30
7	N.D.	N.D.	6.64	5.30	5.30	5.30	5.85
9	N.D.	N.D.	6.48	5.30	5.30	5.30	5.30
10	N.D.	N.D.	5.87	5.30	5.30	5.30	5.30
11	N.D.	N.D.	6.20	5.30	5.30	5.30	5.30
17	5.88	6.74	6.50	5.30	5.30	5.30	5.32
19	5.54	6.55	6.42	5.30	5.30	5.30	6.04
20	6.73	5.93	6.37	5.30	5.30	5.37	5.94
21	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
22	6.56	5.99	6.39	5.30	5.30	5.41	5.98
23	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.

Table 15 continued

Compound	Receptor (pKi)							
25	5.82	5.99	5.35	5.30	5.30	5.39	5.62	
26	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	
27	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	

EXPERIMENTAL DISCUSSION

[0238] In order to isolate new NPY receptor subtypes applicants choose an expression cloning approach where a functional receptor is actually detected with exquisite sensitivity on the surface of transfected cells, using a highly specific iodinated ligand. Using this strategy, applicants have identified a rat hypothalamic cDNA encoding a novel Y-type receptor (Y5). The fact that applicants had to screen 3.5 x 10^6 independent clones with a 2.7 kb average insert size to find two clones reveals either a very strong bias against Y5 cDNA cloning in the cDNA library construction procedure or that the Y5 mRNA is expressed at very low levels in rat hypothalamic tissue. The longest reading frame in the rat Y5 cDNA (CG-18) encodes a 456 amino acid protein with an estimated molecular weight of 50.1 kD. Given there are two N-linked glycosylation site in the amino terminus, the apparent molecular weight could be slightly higher. Applicants have isolated the human Y5 homolog from a human hippocampal cDNA library. The longest reading frame in the human Y5 cDNA (CG-19) encodes a 455 amino acid protein with an estimated molecular weight of 50 kD. The human Y5 receptor is one amino acid shorter than the rat Y5 and shows significant amino acid differences both in the N-terminal and the middle of the third intracellular loop portions of the protein. The seven transmembrane domains and the extracellular loops, however, are virtually identical and the protein motifs found in both species homologs are identical. Both human and rat Y5 receptors carry a large number of potential phosphorylation sites in their second and third intra- cellular loops which could be involved in the regulation of their functional characteristics.

[0239] The rat and human Y5 receptors both carry a leucine zipper in the first putative transmembrane domain. In such a structure, it has been proposed that segments containing periodic arrays of leucine residues exist in an alpha-helical conformation. The leucine side chains extending from one alpha-helix interact with those from a similar alpha helix of a second polypeptide, facilitating dimerization by the formation of a coiled coil (O'Shea et al, 1989). Usually, such patterns are associated with nuclear DNA binding protein like c-myc, c-fos and c-jun, but it is possible that in

some proteins the leucine repeat simply facilitates dimerization and has little to do with positioning a DNA-binding region. Further evidence supporting the idea that dimerization of specific seven transmembrane receptors can occur comes from coexpression studies with muscarinic/adrenergic receptors where intermolecular "cross-talk" between chimeric G-protein coupled receptors has been described (Maggio et al., 1993). The tyrosine phosphorylation site found in the middle of this leucine zipper in transmembrane domain one (TM I) could be involved in regulating dimerization of the Y5 receptor. The physiological significance of G-protein coupled receptor dimerization remains to be elucidated but by analogy with peptide hormone receptors oligomerization, it could be involved in receptor activation and signal transduction (Wells, 1994).

[0240] The nucleotide and amino acid sequence analysis of Y5 (rat and human) reveals low identity levels with all 7 TM receptors including the Y1, Y2 and Y4 receptors, even in the transmembrane domains which are usually highly conserved within receptor subfamilies. Applicants have named CG-18 and CG-19 "Y5" receptors because of their unique amino acid sequence (87.2% identical with each other, ≤ 42% identical with the TM regions of previously cloned "Y" receptor subtypes) and pharmacological profile. The name is not biased toward any one member of the pancreatic polypeptide family. The "Y" has its roots in the original classification of Y1 and Y2 receptor subtypes (Wahlestedt et al., 1987). The letter reflects the conservation in pancreatic polypeptide family members of the C-terminal tyrosine, described as "Y" in the single letter amino acid code. The number is the next available in the Y-type series, position number three having been reserved for the pharmacologically defined Y3 receptor. Applicants note that the cloned human Y1 receptor was introduced by Larhammar and co-workers as a "human neuropeptide Y/peptide YY receptor of the Y1 type" (Larhammar et al., 1992). Similarly, the novel clones described herein can be described as rat and human neuropeptide Y/peptide YY receptors of the Y5 type.

[0241] The rat hypothalamic Y5 receptor displays a very similar pharmacological profile to the pharmacologically described "atypical" Y1 receptor thought to mediate NPY-induced food intake in rat hypothalamus. Both the Y5 receptor and the "feeding receptor" display a preference for NPY and PYY-like analogs, a sensitivity to N-terminal peptide deletion, and a tolerance for Pro^{34}. Each would be considered Y1-like except for the anomalous ability of NPY_{2-36} to bind and activate as well as NPY. Each appears to be sensitive to changes in the mid-region of the peptide ligand. For example, a study by Kalra and colleagues (1991) indicated that replacement of the NPY midregion by an amino-octanoic chain to produce NPY_{1-4}-Aca-$_{25-36}$ dramatically reduced activity in a feeding behavioral assay. Likewise, applicants note that the robust difference in human PP binding (K_i = 5.0 nM) and rat PP binding (K_1 = 230) to the rat Y5 receptor can be attributed to a series of 8 amino acid changes between residues 6-30 in the peptide ligands, with human PP bearing the closer resemblance to human NPY. Note also that FLRFamide, a structural analog of the FMRFamide peptide which is reported to stimulate feeding in rats, was able to bind and activate the rat Y5 receptor albeit at relatively high concentrations (Orosco, et al., 1989). These matching profiles, combined with a selective activation of the rat Y5 by the reported feeding "modulator" [D-Trp^{32}]NPY, support the identity of the rat Y5 as the "feeding receptor" first proposed to explain NPY-induced feeding in rat hypothalamus. That the human Y5 receptor has a pharmacological profile like that of the rat Y5 in both binding and functional assays suggests that the two receptors may have similar functions in vivo.

[0242] The distribution of Y5 mRNA in rat brain further extends the argument for a role of Y5 receptors in feeding behavior. The anatomical locus of the feeding response, for example, has been suggested to reside at least in part in the paraventricular hypothalamic nucleus (PVN) and also in the lateral hypothalamus, two places where Y5 mRNA was detected in abundance. Post-synaptic localization of the Y5 receptor in both of these regions can regulate the response to endogenously released NPY *in vivo*. The paraventricular nucleus receives projections from NPY-containing neurons in the arcuate nucleus, another region where Y5 mRNA was detected. This indicates a pre-synaptic role for the Y5 receptor in the control of NPY release via the arcuatoparaventricular projection, and consequently in the control of feeding behavior. The localization of the Y5 mRNA in the midline thalamic nuclei is also important. The paraventricular thalamic nucleus/centromedial nucleus complex projects heavily to the paraventricular hypothalamus and to the amygdala. As such, the Y5 receptor is a substrate for the emotional aspect of appetitive behaviors.

[0243] Y5 receptors are highly attractive targets for appetite and weight control based on several lines of research (Sahu and Kalra, 1993). NPY is the most potent stimulant of feeding behavior yet described (Clark et al., 1984; Levine and Morley, 1984; Stanley and Leibowitz, 1984). Direct injection of NPY into the hypothalamus of rats can increase food intake ~ 10-fold over a 4-hour period (Stanley et al., 1992). NPY-stimulated rats display a preference for carbohydrates over protein and fat (Stanley et al., 1985). Interestingly, NPY and NPY mRNA are increased in food-deprived rats (Brady et al., 1990; O' Shea and Gundlach, 1991) and also in rats which are genetically obese (Sanacora et al., 1990) or made diabetic by treatment with streptozotocin (White et al., 1990). One potential explanation is that NPY, a potent stimulant of feeding behavior in normal rats, is disregulated in the overweight or diabetic animal so that food intake is increased, accompanied by obesity. The physiological stress of obesity increases the risk for health problems such as cardiovascular malfunction, osteoarthritis, and hyperinsulinemia, together with a worsened prognosis for adult-onset diabetes. A nonpeptide antagonist targeted to the Y5 receptor could therefore be effective as a way to control not only appetite and body weight but an entire range of obesity- and diabetes-related disorders (Dryden et al., 1994).

There is also neurochemical evidence to suggest that NPY-mediaced functions are disregulated in eating disorders such as bulimia and anorexia nervosa, so that they too could be responsive to treatment by a Y5-selective drug. It has been proposed, for example, that food intake in NPY-stimulated rats mimics the massive food consumption associated with binge eating in bulimia (Stanley, 1993). CSF levels of PYY but not NPY were elevated in bulimic patients who abstained from binging, and then diminished when binging was allowed (Berrettini et al., 1988). Conversely, NPY levels were elevated in underweight anorectic patients and then diminished as body weight was normalized (Kaye et al., 1990).

[0244] As described above, the human and rat *in vitro* expression models were used in combination to screen for compounds intended to modulate NPY-dependent feeding behavior. Using this approach, applicants have discovered several compounds which inhibit feeding behavior in animal models, which should lead to additional drug discoveries. The compounds according to the present invention inhibit food intake in Zucker obese rats in a range especially of about 0.01 to about 100 mg/kg after oral, intraperitoneal or intravenous administration.

[0245] The Y5 pharmacological profile further offers a new standard by which to review the molecular basis of all NPY-dependent processes; examples are listed in Table 11. Such an exercise suggests that the Y5 receptor is likely to have a physiological significance beyond feeding behavior. It has been reported, for example, that a Y-type receptor can regulate luteinizing hormone releasing hormone (LHRH) release from the median eminence of steroid-primed rats in vitro with an atypical Y1 pharmacological profile. NPY, NPY_{2-36}, and LP-NPY were all effective at 1uM but deletion of as few as four amino acids from the N-terminus of NPY destroyed biological activity. The Y5 may therefore represent a therapeutic target for sexual or reproductive disorders. Preliminary in situ hybridization of rat Y5 mRNA in hippocampus and elsewhere further suggest that additional roles will be uncovered, for example, in the regulation of memory. It is worth while considering that the Y5 is so similar in pharmacological profile to the other Y-type receptors that it may have been overlooked among a mixed population of Y1, Y2 and Y4 receptors. Certain functions now associated with these subtypes could therefore be reassigned to Y5 as our pharmacological tools grow more sophisticated (Table 18). By offering new insight into NPY receptor pharmacology, the Y5 thereby provides a greater clarity and focus in the field of drug design.

TABLE 17:

Pathophysiological Conditions Associated With NPY		
The following pathological conditions have been linked to either 1) application of exogenous NPY, or 2) changes in levels of endogenous NPY.		
1	obesity	Sahu and Kalra, 1993
2	eating disorders (anorexia and bulimia nervosa)	Stanley, 1993
3	sexual/reproduct ive function	Clark, 1994
4	depression	Heilig and Weiderlov, 1990
5	anxiety	Wahlestedt et al., 1993
6	cocaine addiction	Wahlestedt et al., 1991
7	gastric ulcer	Penner et al., 1993
8	memory loss	Morley and Flood, 1990
9	pain	Hua et al., 1991
10	epileptic seizure	Rizzi et al., 1993
11	hypertension	Zukowska-Grojec et al., 1993
12	subarachnoid hemorrhage	Abel et al., 1988
13	shock	Hauser et al., 1993
14	circadian rhythm	Albers and Ferris, 1984
15	nasal congestion	Lacroix et al., 1988
16	diarrhea	Cox and Cuthbert, 1990
17	neurogenic voiding dysfunction	Zoubek et al., 1993

[0246] A successful strategy for the design of a Y5-receptor based drug or for any drug targeted to single G protein-coupled receptor subtype involves the screening of candidate compounds 1) in radioligand binding assays so as to

detect affinity for cross-reactive G protein-coupled receptors, and 2) in physiological assays so as to detect undesirable side effects. In the specific process of screening for a Y5-selective drug, the receptor subtypes most likely to cross-react and therefore most important for radioligand binding screens include the other "Y-type" receptors, Y1, Y2, Y3, and Y4. Cross-reactivity between the Y5 and any of the other subtypes could result in potential complications as suggested by the pathophysiological indications listed in Table 17. In designing a Y5 antagonist for obesity and appetite control, for example, it is important not to design a Y1 antagonist resulting in hypertension or increased anxiety, a Y2 antagonist resulting in memory loss, or a Y4 antagonist resulting in increased appetite.

TABLE 18:

Y-Type Receptor Indications			
Y-type Receptor Indications	Receptor Subtype	Drug Activity	Reference
obesity, appetite disorder	atypical Y1	antagonist	Sahu and Kalra, 1993
adult onset diabetes	atypical Y1	antagonist	Sahu and Kalra, 1993
bulimia nervosa	atypical Y1	antagonist	Stanley, 1993
pheochromoc ytoma-induced hypertensio n	Y1	antagonist	Grouzman et al., 1989
subarachnoi d hemorrhage	Y1	antagonist	Abel et al., 1988
neurogenic vascular hypertrophy	Y1 Y2	antagonist antagonist	Zukowska-Grojec et al., 1993
epileptic seizure	Y2	antagonist	Rizzi et al., 1993
hypertensio n: central, peripheral regulation	peripheral Y1 central Y3 central Y2	antagonist agonist antagonist	Grundemar and Hakanson, 1993 Barraco et al., 1991
obesity, appetite disorder	Y4 or PP	agonist	Malaisse-Lagae et al., 1977
anorexia nervosa	atypical Y1	agonist	Berrettin i et al., 1988
anxiety	Y1	agonist	Wahlested t et al., 1993
cocaine addiction	Y1	agonist	Wahlested t et al., 1991
stress-induced gastric ulcer	Y1 Y4 or PP	agonist agonist	Penner et al., 1993
memory loss	Y2	agonist	Morley and Flood, 1990
pain	Y2	agonist	Hua et al., 1991
shock	Y1	agonist	Hauser et al., 1993
sleep disturbance s, jet lag	Y2	not clear	Albers and Ferris, 1984
nasal decongestio n	Y1 Y2	agonist agonist	Lacroix et al., 1988
diarrhea	Y2	agonist	Cox and Cuthbert, 1990

[0247] The cloning of the Y5 receptor from human and rat is especially valuable for receptor characterization based on in situ localization, anti-sense functional knockout, and gene induction. These studies will generate important information related to Y5 receptor function and its therapeutic significance. The cloned Y5 receptor lends itself to mutagenesis studies in which receptor/ligand interactions can be modeled. The Y5 receptor further allows us to investigate the possibility of other Y-type receptors through homology cloning. These could include new receptor subtypes as well as Y5 species homologs for the establishment of experimental animal models with relevance for human pathology. The Y5 receptor therefore represents an enormous opportunity for the development of novel and selective drug therapies, particularly those targeted to appetite and weight control, but also for memory loss, depression, anxiety, gastric ulcer, epileptic seizure, pain, hypertension, subarachnoid hemorrhage, sleeping disturbances, nasal congestion, neurogenic voiding dysfuncion, and diarrhea.

[0248] In particular, the discovery of Y5-slective antagonists which inhibit food intake in rats provides a method of

modifying feeding behavior in a wide variety of vertebrate animals.

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SEQUENCE LISTING

[0323]

(1) GENERAL INFORMATION:

(i) APPLICANT: Synaptic Pharmaceutical Corporation

(ii) TITLE OF INVENTION: METHODS OF MODIFYING FEEDING BEHAVIOR, COMPOUNDS USEFUL IN SUCH METHODS, AND DNA ENCODING A HYPOTHALAMIC ATYPICAL NEUROPEPTIDE Y/PEPTIDE YY RECEPTOR (Y5) AND USES THEREOF

(iii) NUMBER OF SEQUENCES: 12

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: Cooper & Dunham LLP
(B) STREET: 1185 Avenue of the Americas
(C) CITY: New York
(D) STATE: New York
(E) COUNTRY: United States of America
(F) ZIP: 10036

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: White, John P.
(B) REGISTRATION NUMBER: 28,678
(C) REFERENCE/DOCKET NUMBER: 1795/46166-A-PCT

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: (212) 278-0400
(B) TELEFAX: (212) 391-0525

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1501 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:

(A) NAME/KEY: CDS
(B) LOCATION: 61..1432

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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252
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CTT GGC TTT ATG GGC AAT CTA CTT ATT TTA ATG GCT GTT ATG AAA AAG
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Leu Gly Phe Met Gly Asn Leu Leu Ile Leu Met Ala Val Met Lys Lys
65                  70                  75                  80

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348
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                85                  90                  95

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828
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876
Glu Asn Arg Leu Glu Glu Asn Glu Met Ile Asn Leu Thr Leu Gln Pro
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TCC AAA AAG AGC AGG AAC CAG GCA AAA ACC CCC AGC ACT CAA AAG TGG
924
Ser Lys Lys Ser Arg Asn Gln Ala Lys Thr Pro Ser Thr Gln Lys Trp
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1068
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1116
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            340                 345                 350

GAA AGC TCA GAT GCT CAT GAG ATG AGA GTC AAG CGT TCC ATC ACT AGA
1164
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        355                 360                 365

ATA AAA AAG AGA TCT CGA AGT GTT TTC TAC AGA CTG ACC ATA CTG ATA
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Ile Lys Lys Arg Ser Arg Ser Val Phe Tyr Arg Leu Thr Ile Leu Ile
    370                 375                 380

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385                 390                 395                 400

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Tyr Cys Ile Cys His Leu Leu Gly Met Met Ser Cys Cys Leu Asn Pro
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1404
Ile Leu Tyr Gly Phe Leu Asn Asn Gly Ile Lys Ala Asp Leu Arg Ala
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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 457 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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                85                  90                  95

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Cys Val Glu Ser Trp Pro Ser Asp Ser Tyr Arg Ile Ala Phe Thr Ile
    210                 215                 220

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Glu Ser Ser Asp Ala His Glu Met Arg Val Lys Arg Ser Ile Thr Arg
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Ile Lys Lys Arg Ser Arg Ser Val Phe Tyr Arg Leu Thr Ile Leu Ile
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Leu Val Phe Ala Val Ser Trp Met Pro Leu His Val Phe His Val Val
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(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1457 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:

(A) NAME/KEY: CDS
(B) LOCATION: 61..1432

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
GTTTCCCTCT GAATAGATTA ATTTAAAGTA GTCATGTAAT GTTTTTTTGG TTGCTGACAA
 60

ATG TCT TTT TAT TCC AAG CAG GAC TAT AAT ATG GAT TTA GAG CTC GAC
108
Met Ser Phe Tyr Ser Lys Gln Asp Tyr Asn Met Asp Leu Glu Leu Asp
  1               5                  10                  15

GAG TAT TAT AAC AAG ACA CTT GCC ACA GAG AAT AAT ACT GCT GCC ACT
156
Glu Tyr Tyr Asn Lys Thr Leu Ala Thr Glu Asn Asn Thr Ala Ala Thr
            20                  25                  30
```

```
CGG AAT TCT GAT TTC CCA GTC TGG GAT GAC TAT AAA AGC AGT GTA GAT
204
Arg Asn Ser Asp Phe Pro Val Trp Asp Asp Tyr Lys Ser Ser Val Asp
          35                  40                  45

GAC TTA CAG TAT TTT CTG ATT GGG CTC TAT ACA TTT GTA AGT CTT CTT
252
Asp Leu Gln Tyr Phe Leu Ile Gly Leu Tyr Thr Phe Val Ser Leu Leu
     50                  55                  60

GGC TTT ATG GGG AAT CTA CTT ATT TTA ATG GCT CTC ATG AAA AAG CGT
300
Gly Phe Met Gly Asn Leu Leu Ile Leu Met Ala Leu Met Lys Lys Arg
 65                  70                  75                  80

AAT CAG AAG ACT ACG GTA AAC TTC CTC ATA GGC AAT CTG GCC TTT TCT
348
Asn Gln Lys Thr Thr Val Asn Phe Leu Ile Gly Asn Leu Ala Phe Ser
                 85                  90                  95

GAT ATC TTG GTT GTG CTG TTT TGC TCA CCT TTC ACA CTG ACG TCT GTC
396
Asp Ile Leu Val Val Leu Phe Cys Ser Pro Phe Thr Leu Thr Ser Val
            100                 105                 110

TTG CTG GAT CAG TGG ATG TTT GGC AAA GTC ATG TGC CAT ATT ATG CCT
444
Leu Leu Asp Gln Trp Met Phe Gly Lys Val Met Cys His Ile Met Pro
        115                 120                 125

TTT CTT CAA TGT GTG TCA GTT TTG GTT TCA ACT TTA ATT TTA ATA TCA
492
Phe Leu Gln Cys Val Ser Val Leu Val Ser Thr Leu Ile Leu Ile Ser
    130                 135                 140

ATT GCC ATT GTC AGG TAT CAT ATG ATA AAA CAT CCC ATA TCT AAT AAT
540
Ile Ala Ile Val Arg Tyr His Met Ile Lys His Pro Ile Ser Asn Asn
145                 150                 155                 160

TTA ACA GCA AAC CAT GGC TAC TTT CTG ATA GCT ACT GTC TGG ACA CTA
588
Leu Thr Ala Asn His Gly Tyr Phe Leu Ile Ala Thr Val Trp Thr Leu
                165                 170                 175

GGT TTT GCC ATC TGT TCT CCC CTT CCA GTG TTT CAC AGT CTT GTG GAA
636
Gly Phe Ala Ile Cys Ser Pro Leu Pro Val Phe His Ser Leu Val Glu
            180                 185                 190

CTT CAA GAA ACA TTT GGT TCA GCA TTG CTG AGC AGC AGG TAT TTA TGT
684
Leu Gln Glu Thr Phe Gly Ser Ala Leu Leu Ser Ser Arg Tyr Leu Cys
        195                 200                 205

GTT GAG TCA TGG CCA TCT GAT TCA TAC AGA ATT GCC TTT ACT ATC TCT
732
Val Glu Ser Trp Pro Ser Asp Ser Tyr Arg Ile Ala Phe Thr Ile Ser
    210                 215                 220

TTA TTG CTA GTT CAG TAT ATT CTG CCC TTA GTT TGT CTT ACT GTA AGT
780
Leu Leu Leu Val Gln Tyr Ile Leu Pro Leu Val Cys Leu Thr Val Ser
225                 230                 235                 240

CAT ACA AGT GTC TGC AGA AGT ATA AGC TGT GGA TTG TCC AAC AAA GAA
828
His Thr Ser Val Cys Arg Ser Ile Ser Cys Gly Leu Ser Asn Lys Glu
                245                 250                 255
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AAC AGA CTT GAA GAA AAT GAG ATG ATC AAC TTA ACT CTT CAT CCA TCC
876
Asn Arg Leu Glu Glu Asn Glu Met Ile Asn Leu Thr Leu His Pro Ser
            260                 265                 270

AAA AAG AGT GGG CCT CAG GTG AAA CTC TCT GGC AGC CAT AAA TGG AGT
924
Lys Lys Ser Gly Pro Gln Val Lys Leu Ser Gly Ser His Lys Trp Ser
        275                 280                 285

TAT TCA TTC ATC AAA AAA CAC AGA AGA AGA TAT AGC AAG AAG ACA GCA
972
Tyr Ser Phe Ile Lys Lys His Arg Arg Arg Tyr Ser Lys Lys Thr Ala
    290                 295                 300

TGT GTG TTA CCT GCT CCA GAA AGA CCT TCT CAA GAG AAC CAC TCC AGA
1020
Cys Val Leu Pro Ala Pro Glu Arg Pro Ser Gln Glu Asn His Ser Arg
305                 310                 315                 320

ATA CTT CCA GAA AAC TTT GGC TCT GTA AGA AGT CAG CTC TCT TCA TCC
1068
Ile Leu Pro Glu Asn Phe Gly Ser Val Arg Ser Gln Leu Ser Ser Ser
                325                 330                 335

AGT AAG TTC ATA CCA GGG GTC CCC ACT TGC TTT GAG ATA AAA CCT GAA
1116
Ser Lys Phe Ile Pro Gly Val Pro Thr Cys Phe Glu Ile Lys Pro Glu
            340                 345                 350

GAA AAT TCA GAT GTT CAT GAA TTG AGA GTA AAA CGT TCT GTT ACA AGA
1164
Glu Asn Ser Asp Val His Glu Leu Arg Val Lys Arg Ser Val Thr Arg
        355                 360                 365

ATA AAA AAG AGA TCT CGA AGT GTT TTC TAC AGA CTG ACC ATA CTG ATA
1212
Ile Lys Lys Arg Ser Arg Ser Val Phe Tyr Arg Leu Thr Ile Leu Ile
    370                 375                 380

TTA GTA TTT GCT GTT AGT TGG ATG CCA CTA CAC CTT TTC CAT GTG GTA
1260
Leu Val Phe Ala Val Ser Trp Met Pro Leu His Leu Phe His Val Val
385                 390                 395                 400

ACT GAT TTT AAT GAC AAT CTT ATT TCA AAT AGG CAT TTC AAG TTG GTG
1308
Thr Asp Phe Asn Asp Asn Leu Ile Ser Asn Arg His Phe Lys Leu Val
                405                 410                 415

TAT TGC ATT TGT CAT TTG TTG GGC ATG ATG TCC TGT TGT CTT AAT CCA
1356
Tyr Cys Ile Cys His Leu Leu Gly Met Met Ser Cys Cys Leu Asn Pro
            420                 425                 430

ATT CTA TAT GGG TTT CTT AAT AAT GGG ATT AAA GCT GAT TTA GTG TCC
1404
Ile Leu Tyr Gly Phe Leu Asn Asn Gly Ile Lys Ala Asp Leu Val Ser
        435                 440                 445

CTT ATA CAC TGT CTT CAT ATG TAA TAA TTCTCACTGT TTACCAAGGA
1452
Leu Ile His Cys Leu His Met  *   *
    450                 455

AAGAAC
1457
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 457 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Met Ser Phe Tyr Ser Lys Gln Asp Tyr Asn Met Asp Leu Glu Leu Asp
 1               5                  10                  15

Glu Tyr Tyr Asn Lys Thr Leu Ala Thr Glu Asn Asn Thr Ala Ala Thr
            20                  25                  30

Arg Asn Ser Asp Phe Pro Val Trp Asp Asp Tyr Lys Ser Ser Val Asp
        35                  40                  45

Asp Leu Gln Tyr Phe Leu Ile Gly Leu Tyr Thr Phe Val Ser Leu Leu
    50                  55                  60

Gly Phe Met Gly Asn Leu Leu Ile Leu Met Ala Leu Met Lys Lys Arg
65                  70                  75                  80

Asn Gln Lys Thr Thr Val Asn Phe Leu Ile Gly Asn Leu Ala Phe Ser
                85                  90                  95

Asp Ile Leu Val Val Leu Phe Cys Ser Pro Phe Thr Leu Thr Ser Val
            100                 105                 110

Leu Leu Asp Gln Trp Met Phe Gly Lys Val Met Cys His Ile Met Pro
        115                 120                 125

Phe Leu Gln Cys Val Ser Val Leu Val Ser Thr Leu Ile Leu Ile Ser
    130                 135                 140

Ile Ala Ile Val Arg Tyr His Met Ile Lys His Pro Ile Ser Asn Asn
145                 150                 155                 160

Leu Thr Ala Asn His Gly Tyr Phe Leu Ile Ala Thr Val Trp Thr Leu
                165                 170                 175

Gly Phe Ala Ile Cys Ser Pro Leu Pro Val Phe His Ser Leu Val Glu
            180                 185                 190

Leu Gln Glu Thr Phe Gly Ser Ala Leu Leu Ser Ser Arg Tyr Leu Cys
        195                 200                 205

Val Glu Ser Trp Pro Ser Asp Ser Tyr Arg Ile Ala Phe Thr Ile Ser
    210                 215                 220

Leu Leu Leu Val Gln Tyr Ile Leu Pro Leu Val Cys Leu Thr Val Ser
225                 230                 235                 240

His Thr Ser Val Cys Arg Ser Ile Ser Cys Gly Leu Ser Asn Lys Glu
                245                 250                 255

Asn Arg Leu Glu Glu Asn Glu Met Ile Asn Leu Thr Leu His Pro Ser
            260                 265                 270

Lys Lys Ser Gly Pro Gln Val Lys Leu Ser Gly Ser His Lys Trp Ser
        275                 280                 285

Tyr Ser Phe Ile Lys Lys His Arg Arg Arg Tyr Ser Lys Lys Thr Ala
    290                 295                 300

Cys Val Leu Pro Ala Pro Glu Arg Pro Ser Gln Glu Asn His Ser Arg
305                 310                 315                 320

Ile Leu Pro Glu Asn Phe Gly Ser Val Arg Ser Gln Leu Ser Ser Ser
```

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                 325                 330                 335

Ser Lys Phe Ile Pro Gly Val Pro Thr Cys Phe Glu Ile Lys Pro Glu
            340                 345                 350

Glu Asn Ser Asp Val His Glu Leu Arg Val Lys Arg Ser Val Thr Arg
        355                 360                 365

Ile Lys Lys Arg Ser Arg Ser Val Phe Tyr Arg Leu Thr Ile Leu Ile
    370                 375                 380

Leu Val Phe Ala Val Ser Trp Met Pro Leu His Leu Phe His Val Val
385                 390                 395                 400

Thr Asp Phe Asn Asp Asn Leu Ile Ser Asn Arg His Phe Lys Leu Val
                405                 410                 415

Tyr Cys Ile Cys His Leu Leu Gly Met Met Ser Cys Cys Leu Asn Pro
            420                 425                 430

Ile Leu Tyr Gly Phe Leu Asn Asn Gly Ile Lys Ala Asp Leu Val Ser
        435                 440                 445

Leu Ile His Cys Leu His Met  *   *
    450                 455
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1054 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE :

(A) NAME/KEY: CDS
(B) LOCATION: 3..1004

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
TC ATG TGT CAC ATT ATG CCT TTT CTT CAA TGT GTG TCA GTT CTG GTT
 47
   Met Cys His Ile Met Pro Phe Leu Gln Cys Val Ser Val Leu Val
     1               5                  10                  15

TCA ACT TTA ATT CTA ATA TCA ATT GCC ATT GTC AGG TAT CAT ATG ATC
 95
Ser Thr Leu Ile Leu Ile Ser Ile Ala Ile Val Arg Tyr His Met Ile
                20                  25                  30

AAG CAT CCT ATA TCT AAC AAT TTA ACA GCA AAC CAT GGC TAC TTC CTG
 143
Lys His Pro Ile Ser Asn Asn Leu Thr Ala Asn His Gly Tyr Phe Leu
            35                  40                  45

ATT GCT ACT GTC TGG ACA CTA GGT TTT GCG ATT TGT TCT CCC CTT CCA
 191
Ile Ala Thr Val Trp Thr Leu Gly Phe Ala Ile Cys Ser Pro Leu Pro
         50                  55                  60

GTG TTT CAC AGT CTG GTG GAA CTT CAG GAA ACA TTT GAC TCC GCA TTG
 239
Val Phe His Ser Leu Val Glu Leu Gln Glu Thr Phe Asp Ser Ala Leu
     65                  70                  75

CTG AGC AGC AGG TAT TTA TGT GTT GAG TCG TGG CCA TCT GAT TCG TAC
 287
```

```
Leu Ser Ser Arg Tyr Leu Cys Val Glu Ser Trp Pro Ser Asp Ser Tyr
 80                  85                  90                  95

AGA ATC GCT TTT ACT ATC TCT TTA TTG CTA GTC CAG TAT ATT CTT CCC
 335
Arg Ile Ala Phe Thr Ile Ser Leu Leu Leu Val Gln Tyr Ile Leu Pro
                100                 105                 110

TTG GTG TGT CTA ACT GTG AGC CAT ACC AGT GTC TGC AGG AGT ATA AGC
 383
Leu Val Cys Leu Thr Val Ser His Thr Ser Val Cys Arg Ser Ile Ser
            115                 120                 125

TGC GGG TTG TCC AAC AAA GAA AAC AAA CTG GAA GAA AAC GAG ATG ATC
 431
Cys Gly Leu Ser Asn Lys Glu Asn Lys Leu Glu Glu Asn Glu Met Ile
        130                 135                 140

AAC TTA ACT CTT CAA CCA TTC AAA AAG AGT GGG CCT CAG GTG AAA CTT
 479
Asn Leu Thr Leu Gln Pro Phe Lys Lys Ser Gly Pro Gln Val Lys Leu
    145                 150                 155

TCC AGC AGC CAT AAA TGG AGC TAT TCA TTC ATC AGA AAA CAC AGG AGA
 527
Ser Ser Ser His Lys Trp Ser Tyr Ser Phe Ile Arg Lys His Arg Arg
160                 165                 170                 175

AGG TAC AGC AAG AAG ACG GCG TGT GTC TTA CCT GCT CCA GCA AGA CCT
 575
Arg Tyr Ser Lys Lys Thr Ala Cys Val Leu Pro Ala Pro Ala Arg Pro
                180                 185                 190

CCT CAA GAG AAC CAC TCA AGA ATG CTT CCA GAA AAC TTT GGT TCT GTA
 623
Pro Gln Glu Asn His Ser Arg Met Leu Pro Glu Asn Phe Gly Ser Val
            195                 200                 205

AGA AGT CAG CAT TCT TCA TCC AGT AAG TTC ATA CCG GGG GTC CCC ACC
 671
Arg Ser Gln His Ser Ser Ser Ser Lys Phe Ile Pro Gly Val Pro Thr
        210                 215                 220

TGC TTT GAG GTG AAA CCT GAA GAA AAC TCG GAT GTT CAT GAC ATG AGA
 719
Cys Phe Glu Val Lys Pro Glu Glu Asn Ser Asp Val His Asp Met Arg
    225                 230                 235

GTA AAC CGT TCT ATC ATG AGA ATC AAA AAG AGA TCC CGA AGT GTT TTC
 767
Val Asn Arg Ser Ile Met Arg Ile Lys Lys Arg Ser Arg Ser Val Phe
240                 245                 250                 255

TAT AGA CTA ACC ATA CTG ATA CTA GTG TTT GCC GTT AGC TGG ATG CCA
 815
Tyr Arg Leu Thr Ile Leu Ile Leu Val Phe Ala Val Ser Trp Met Pro
                260                 265                 270

CTA CAC CTT TTC CAT GTG GTA ACT GAT TTT AAT GAC AAC CTC ATT TCA
 863
Leu His Leu Phe His Val Val Thr Asp Phe Asn Asp Asn Leu Ile Ser
            275                 280                 285

AAC AGG CAT TTC AAA TTG GTG TAT TGC ATT TGT CAT TTG TTA GGC ATG
 911
Asn Arg His Phe Lys Leu Val Tyr Cys Ile Cys His Leu Leu Gly Met
        290                 295                 300

ATG TCC TGT TGT CTT AAT CCT ATT CTG TAT GGT TTT CTC AAT AAT GGG
 959
```

```
Met Ser Cys Cys Leu Asn Pro Ile Leu Tyr Gly Phe Leu Asn Asn Gly
    305                 310                 315

ATC AAA GCT GAT TTA ATT TCC CTT ATA CAG TGT CTT CAT ATG TCA
1004
Ile Lys Ala Asp Leu Ile Ser Leu Ile Gln Cys Leu His Met Ser
320                 325                 330

TAATTATTAA TGTTTACCAA GGAGACAACA AATGTTGGGA TCGTCTAAAA
1054
```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 334 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
Met Cys His Ile Met Pro Phe Leu Gln Cys Val Ser Val Leu Val Ser
 1               5                  10                  15

Thr Leu Ile Leu Ile Ser Ile Ala Ile Val Arg Tyr His Met Ile Lys
            20                  25                  30

His Pro Ile Ser Asn Asn Leu Thr Ala Asn His Gly Tyr Phe Leu Ile
        35                  40                  45

Ala Thr Val Trp Thr Leu Gly Phe Ala Ile Cys Ser Pro Leu Pro Val
    50                  55                  60

Phe His Ser Leu Val Glu Leu Gln Glu Thr Phe Asp Ser Ala Leu Leu
65                  70                  75                  80

Ser Ser Arg Tyr Leu Cys Val Glu Ser Trp Pro Ser Asp Ser Tyr Arg
                85                  90                  95

Ile Ala Phe Thr Ile Ser Leu Leu Leu Val Gln Tyr Ile Leu Pro Leu
            100                 105                 110

Val Cys Leu Thr Val Ser His Thr Ser Val Cys Arg Ser Ile Ser Cys
        115                 120                 125

Gly Leu Ser Asn Lys Glu Asn Lys Leu Glu Glu Asn Glu Met Ile Asn
    130                 135                 140

Leu Thr Leu Gln Pro Phe Lys Lys Ser Gly Pro Gln Val Lys Leu Ser
145                 150                 155                 160

Ser Ser His Lys Trp Ser Tyr Ser Phe Ile Arg Lys His Arg Arg Arg
                165                 170                 175

Tyr Ser Lys Lys Thr Ala Cys Val Leu Pro Ala Pro Ala Arg Pro Pro
            180                 185                 190

Gln Glu Asn His Ser Arg Met Leu Pro Glu Asn Phe Gly Ser Val Arg
        195                 200                 205

Ser Gln His Ser Ser Ser Ser Lys Phe Ile Pro Gly Val Pro Thr Cys
    210                 215                 220

Phe Glu Val Lys Pro Glu Glu Asn Ser Asp Val His Asp Met Arg Val
225                 230                 235                 240

Asn Arg Ser Ile Met Arg Ile Lys Lys Arg Ser Arg Ser Val Phe Tyr
                245                 250                 255

Arg Leu Thr Ile Leu Ile Leu Val Phe Ala Val Ser Trp Met Pro Leu
            260                 265                 270

His Leu Phe His Val Val Thr Asp Phe Asn Asp Asn Leu Ile Ser Asn
        275                 280                 285

Arg His Phe Lys Leu Val Tyr Cys Ile Cys His Leu Leu Gly Met Met
    290                 295                 300

Ser Cys Cys Leu Asn Pro Ile Leu Tyr Gly Phe Leu Asn Asn Gly Ile
305                 310                 315                 320

Lys Ala Asp Leu Ile Ser Leu Ile Gln Cys Leu His Met Ser
                325                 330
```

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

TGGATCAGTG GATGTTTGGC AAAG
24

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GTCTGTAGAA AACACTTCGA GATCTCTT
28

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CTTCCAGTGT TTCACAGTCT GGTGG
25

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

```
CTGAGCAGCA GGTATTTATG TGTTG
 25
```

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

```
CTGGATGAAG AATGCTGACT TCTTAGAG
 28
```

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

```
TTCTTGAGTG GTTCTCTTGA GGAGG
 25
```

Claims

1. A nucleic acid encoding a mammalian Y5 receptor, said nucleic acid containing:

   (a) a nucleotide sequence encoding the amino acid sequence shown in Figure 6; or

   (b) a nucleotide sequence encoding the amino acid sequence shown in Figure 4; or

   (c) a nucleotide sequence encoding the amino acid sequence shown in Figure 15; or

   (d) a nucleotide sequence which encodes a Y5 receptor in a mammal other than a human, a rat, or a canine and which hybridizes under suitable conditions to any one of (a) to (c);

   wherein the receptor is **characterized by** (1) a pharmacological profile characteristic of the human Y5 receptor as shown in Table 6 or Table 7; or (2) a pharmacological profile characteristic of the rat Y5 receptor as shown in Table 4 or Table 5.

2. The nucleic acid of claim 1, which is DNA or RNA, wherein if RNA, the RNA is preferably mRNA.

3. The nucleic acid of claim 2, which is cDNA or genomic DNA.

4. The nucleic acid of claim 1, wherein the nucleic acid encodes a Y5 receptor **characterized by** an amino acid sequence in each of transmembrane regions I-VII which is identical to the amino acid sequence in the corresponding transmembrane region of the human Y5 receptor shown in Figure 8.

5. A purified Y5 receptor protein encoded by the nucleic acid molecule of any one of claims 1 to 4.

6. A vector comprising the nucleic acid of any one of claims 1 to 4.

7. A vector of claim 6 adapted for expression in a host cell which comprises the regulatory elements necessary for expression of the nucleic acid in the host cell operatively linked to the nucleic acid encoding a Y5 receptor so as to permit expression thereof.

8. The vector of claim 7, wherein the host cell is a bacterial, yeast, insect or mammalian cell.

9. The vector of claim 8 which is a baculovirus or a plasmid.

10. The plasmid of claim 9 which is pcEXV-hY5 (ATCC Accession No. 75943) or pcEXV-rY5 (ATCC Accession No. 75944).

11. A host cell comprising the vector of any one of claims 6 to 10.

12. The host cell of claim 11 which is a bacterial, yeast, insect, or mammalian cell.

13. The host cell of claim 11 or 12 which is non-neuronal in origin.

14. The host cell of claim 13, which is a COS-7 cell, a 293 human embryonic kidney cell, a NIH-3T3 cell or an LM(tk-) cell.

15. The 293 human embryonic kidney cell of claim 14 which is 293-rY5-14 (ATCC Accession No. CRL 11757).

16. The NIH-3T3 cell of claim 14 which is N-hY5-8 (ATCC Accession No. CRL-11994).

17. The LM(tk-) cell of claim 14 which is L-hY5-7 (ATCC Accession No. CRL-11995).

18. The host cell of claim 12, wherein the insect cell is an Sf9 cell or an Sf21 cell.

19. A method of preparing the purified human, rat or canine Y5 receptor of claim 5 which comprises:

a. constructing a vector adapted for expression in a cell which comprises the regulatory elements necessary for the expression of nucleic acid in the cell operatively linked to the nucleic acid encoding the human, rat or canine Y5 receptor as to permit expression thereof, wherein the cell is selected from the group consisting of bacterial cells, yeast cells, insect cells and mammalian cells;

b. inserting the vector of step a in a suitable host cell;

c. incubating the cells of step b under conditions allowing the expression of the human, rat or canine Y5 receptor;

d. recovering the receptor so produced; and

e. purifying the receptor so recovered, thereby preparing a human, rat or canine Y5 receptor.

20. A method of preparing a purified Y5 receptor which comprises:

(a) placing the host cell of any one of claims 11 to 18 in suitable conditions permitting the production of the

Y5 receptor,

(b) recovering the receptor so produced by the host cell; and,

(c) purifying the receptor so recovered.

21. A purified Y5 receptor prepared by the method of claim 19 or 20.

22. A membrane preparation isolated from the host cell of any one of claims 11 to 18 which host cell does not naturally express a Y5 receptor.

23. An antibody capable of binding to the receptor of claim 21.

24. An antibody capable of competitively inhibiting the binding to a Y5 receptor of the antibody of claim 23.

25. The antibody of claim 23 or 24 which is a monoclonal antibody.

26. The monoclonal antibody of claim 25 which is directed to an epitope of a Y5 receptor present on the surface of a Y5 receptor expressing cell.

27. A pharmaceutical composition which comprises the antibody of any one of claims 25 to 26 and a pharmaceutically acceptable carrier.

28. A process for determining whether a chemical compound specifically binds to a Y5 receptor which comprises contacting host cells of claim 11, or the membrane preparation of claim 22, with the chemical compound under conditions suitable for binding, and detecting specific binding of the chemical compound to the Y5 receptor.

29. A process involving competitive binding for identifying a chemical compound which specifically binds to a Y5 receptor, which comprises separately contacting host cells of claim 11, or the membrane preparation of claim 22, with both the chemical compound and a second chemical compound known to bind to the Y5 receptor, and with only the second chemical compound, under conditions suitable for binding of compounds, and detecting specific binding of the chemical compound to the Y5 receptor, a decrease in binding of the second chemical compound to the Y5 receptor in the presence of the chemical compound indicating that the chemical compound binds to the Y5 receptor.

30. A process involving competitive binding to identify a chemical compound which specifically binds to a Y5 receptor, which comprises separately contacting host cells of claim 11, or the membrane preparation of claim 22, with both a chemical compound known to bind specifically to the Y5 receptor and a plurality of chemical compounds not known to bind specifically to the Y5 receptor, and with only the chemical compound known to bind to the Y5 receptor, under conditions suitable for binding of compounds, detecting specific binding of the plurality of chemical compounds, a decrease in the binding of the chemical compound known to bind to the Y5 receptor in the presence of the plurality of chemical compounds indicating that at least a chemical compound included in the plurality of chemical compounds binds to the Y5 receptor, and separately detecting the binding of each chemical compound included in the plurality of compounds to the Y5 receptor.

31. A process for determining whether a chemical compound is a Y5 receptor agonist, which comprises contacting host cells of claim 11, or the membrane preparation of claim 22, with the chemical compound under conditions permitting activation of the Y5 receptor, and detecting an increase in Y5 receptor activity, so as to thereby determine whether the chemical compound is a Y5 receptor agonist.

32. A process for determining whether a chemical compound specifically binds to and activates a Y5 receptor, which comprises contacting host cells of claim 11, or the membrane preparation of claim 22, with the chemical compound under conditions suitable for activation of the Y5 receptor, and measuring a second messenger response in the presence and in the absence of the chemical compound, a change in second messenger response in the presence of the chemical compound indicating that the chemical compound activates the Y5 receptor.

33. A process for determining whether a chemical compound specifically binds to and activates a Y5 receptor, which comprises contacting host cells of claim 11, or the membrane preparation of claim 22, with a plurality of chemical

compounds not known to bind to and activate the Y5 receptor, under conditions suitable for activation of the Y5 receptor, measuring a second messenger response in the presence and in the absence of the plurality of chemical compounds, a change in the second messenger response in the presence of the plurality of chemical compounds indicating that at least a chemical compound in the plurality of chemical compounds activates the Y5 receptor, and separately determining whether each compound included in the plurality of compounds binds to and activates the Y5 receptor.

34. The process of claim 32 or 33, wherein the second messenger response comprises adenylate cyclase activity and the change in second messenger response is a decrease in adenylate cyclase activity.

35. The process of claim 32 or 33, wherein the second messenger response comprises intracellular calcium concentration and the change in second messenger response is an increase in intracellular calcium concentration.

36. A process for determining whether a chemical compound is a Y5 receptor antagonist, which comprises contacting host cells of claim 11, or the membrane preparation of claim 22, with the chemical compound in the presence of a known Y5 receptor agonist, under conditions permitting the activation of the Y5 receptor, and detecting a decrease in Y5 receptor activity, so as to thereby determine whether the chemical compound is a Y5 receptor antagonist.

37. A process for determining whether a chemical compound specifically binds to and inhibits activation of a Y5 receptor, which comprises separately contacting host cells of claim 11, or the membrane preparation of claim 22, with both the chemical compound and a second chemical compound known to activate the Y5 receptor, and with only the second chemical compound, under conditions suitable for activation of the Y5 receptor, and measuring a second messenger response in the presence of only the second chemical compound and in the presence of both the second chemical compound and the chemical compound, a smaller change in second messenger response in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound indicating that the chemical compound inhibits activation of the Y5 receptor.

38. A process for determining whether a chemical compound specifically binds to and inhibits activation of a Y5 receptor, which comprises separately contacting host cells of claim 11, or the membrane preparation of claim 22, with both a chemical compound known to activate the Y5 receptor and a plurality of chemical compounds not known to inhibit activation of the Y5 receptor, and with only the chemical compound known to activate the Y5 receptor, under conditions suitable for activation of the Y5 receptor, and measuring a second messenger response in the presence of only the chemical compound known to activate the Y5 receptor and in the presence of both the chemical compound known to activate the Y5 receptor and the plurality of chemical compounds, a smaller change in the second messenger response in the presence of both the chemical compound known to activate the Y5 receptor and the plurality of chemical compounds than in the presence of only the chemical compound known to activate the Y5 receptor indicating that at least a chemical compound included in the plurality of chemical compounds inhibits activation of the Y5 receptor and separately determining whether each compound included in the plurality of chemical compounds specifically binds to and inhibits activation of the Y5 receptor.

39. The process of claim 37 or 38, wherein the second messenger response comprises adenylate cyclase activity and the change in second messenger response is a smaller decrease in the level of adenylate cyclase activity in the presence of both the chemical compound and the second chemical compound, or the chemical compound known to activate the Y5 receptor and the plurality of chemical compounds, than in the presence of only the second chemical compound, or the chemical compound known to activate the Y5 receptor.

40. The process of claim 37 or 38, wherein the second messenger response comprises intracellular calcium concentration and the change in second messenger response is a smaller increase in intracellular calcium concentration in the presence of both the chemical compound and the second chemical compound, or the chemical compound known to activate the Y5 receptor and the plurality of chemical compounds, than in the presence of only the second chemical compound, or the chemical compound known to activate the Y5 receptor.

41. A method of detecting the presence of a human Y5 receptor on the surface of a cell in vitro which comprises contacting the cell with the antibody of any one of claims 23 to 26 under conditions permitting binding of the antibody to the receptor, detecting the presence of the antibody bound to the cell, and thereby detecting the presence of a human Y5 receptor on the surface of the cell.

42. A method of preparing a composition which comprises determining whether a chemical compound is a Y5 receptor

agonist using the method of claim 31, separating the chemical compound which has been so determined to be a Y5 receptor agonist from the host cells or the membrane preparation, and placing the chemical compound in a carrier.

43. A method of preparing a composition which comprises determining whether a chemical compound is a Y5 receptor antagonist using the method of claim 36, separating the chemical compound which has been so determined to be a Y5 receptor antagonist from the host cells or the membrane preparation, and placing the chemical compound in a carrier.

Patentansprüche

1. Nucleinsäure, die einen Säuger-Y5-Rezeptor codiert, wobei die Nucleinsäure enthält:

   (a) eine Nucleotidsequenz, die die in Figur 6 gezeigte Aminosäuresequenz codiert; oder
   (b) eine Nucleotidsequenz, die die in Figur 4 gezeigte Aminosäuresequenz codiert; oder
   (c) eine Nucleotidsequenz, die die in Figur 15 gezeigte Aminosäuresequenz codiert; oder
   (d) eine Nucleotidsequenz, die einen Y5-Rezeptor eines Säugers, der kein Mensch ist, einer Ratte oder eines Hundes codiert und die unter geeigneten Bedingungen an eine beliebige Sequenz aus (a) bis (c) hybridisiert;

   wobei der Rezeptor **gekennzeichnet ist durch** (1) ein pharmakologisches Profil, das den menschlichen Y5-Rezeptor kennzeichnet, wie in Tabelle 6 oder 7 gezeigt; oder (2) ein pharmakologisches Profil, das den Y5-Rezeptor der Ratte kennzeichnet, wie in Tabelle 4 oder 5 gezeigt.

2. Nucleinsäure nach Anspruch 1, die DNA oder RNA ist, worin im Fall von RNA die RNA vorzugsweise mRNA ist.

3. Nucleinsäure nach Anspruch 2, die cDNA oder genomische DNA ist.

4. Nucleinsäure nach Anspruch 1, wobei die Nucleinsäure einen Y5-Rezeptor codiert, der **gekennzeichnet ist durch** eine Aminosäuresequenz in jeder der Transmembranregionen I-VII, die zu der Aminosäuresequenz in der entsprechenden Transmembranregion des in Figure 8 gezeigten menschlichen Y5-Rezeptors identisch ist.

5. Gereinigtes Y5-Rezeptor-Protein, codiert durch das Nucleinsäuremolekül nach einem der Ansprüche 1 bis 4.

6. Vektor, der die Nucleinsäure nach einem der Ansprüche 1 bis 4 umfasst.

7. Vektor nach Anspruch 6, der für die Expression in einer Wirtszelle angepasst wurde, umfassend die regulatorischen Elemente, die für die Expression der Nucleinsäure in der Wirtszelle notwendig sind, funktionell mit der Nucleinsäure verknüpft, die den Y5-Rezeptor codiert, um dessen Expression zu erlauben.

8. Vektor nach Anspruch 7, wobei die Wirtszelle eine Bakterien-, Hefe-, Insekten- oder Säugerzelle ist.

9. Vektor nach Anspruch 8, der ein Baculovirus oder ein Plasmid ist.

10. Plasmid nach Anspruch 9, das pcEXV-hY5 (ATCC-Hinterlegungsnummer 75943) oder pcEXV-rY5 (ATCC-Hinterlegungsnummer 75944) ist.

11. Wirtszelle, die den Vektor nach einem der Ansprüche 6 bis 10 umfasst.

12. Wirtszelle nach Anspruch 11, die eine Bakterien-, Hefe-, Insekten- oder Säugerzelle ist.

13. Wirtszelle nach Anspruch 11 oder 12, die nicht-neuronalen Ursprungs ist.

14. Wirtszelle nach Anspruch 13, die eine COS-7-Zelle, eine menschliche embryonale Nierenzelle 293, eine NIH-3T3-Zelle oder eine LM(tk-)-Zelle ist.

15. Menschliche embryonale Nierenzelle 293 nach Anspruch 14, die 293-rY5-14 (ATCC-Hinterlegungsnummer CRL 11757) ist.

16. NIH-3T3-Zelle nach Anspruch 14, die N-hY5-8 (ATCC-Hinterlegungsnummer CRL-11994) ist.

17. LM(tk-)-Zelle nach Anspruch 14, die L-hY5-7 (ATCC-Hinterlegungsnummer CRL-11995) ist.

18. Wirtszelle nach Anspruch 12, worin die Insektenzelle eine Sf9-Zelle oder eine Sf21-Zelle ist.

19. Verfahren zur Herstellung des gereinigten Y5-Rezeptors von Mensch, Ratte oder Hund nach Anspruch 5, welches umfasst:

(a) Konstruieren eines für die Expression in einer Zelle angepassten Vektors, der regulatorische Elemente umfasst, die für die Expression der Nucleinsäure in der Zelle notwendig sind, funktionell verknüpft mit der Nucleinsäure, die den Y5-Rezeptor von Mensch, Ratte oder Hund codiert, um dessen Expression zu erlauben, wobei die Zelle ausgewählt ist aus der Gruppe, bestehend aus Bakterienzellen, Hefezellen, Insektenzellen und Säugerzellen;
(b) Einschleusen des Vektors aus Schritt (a) in eine geeignete Wirtszelle;
(c) Inkubieren der Zellen aus Schritt (b) unter Bedingungen, die die Expression des Y5-Rezeptors von Mensch, Ratte oder Hund erlauben;
(d) Gewinnen des so hergestellten Rezeptors; und
(e) Reinigen des so gewonnen Rezeptors, wodurch ein Y5-Rezeptor von Mensch, Ratte oder Hund hergestellt wird.

20. Verfahren zur Herstellung eines gereinigten Y5-Rezeptors, welches umfasst:

(a) Unterwerfen der Wirtszelle nach einem der Ansprüche 11 bis 18 unter geeignete Bedingungen, die die Herstellung des Y5-Rezeptors erlauben;
(b) Gewinnen des so durch die Wirtszelle erzeugten Rezeptors; und
(c) Reinigen des so gewonnenen Rezeptors.

21. Gereinigter Y5-Rezeptor, hergestellt nach dem Verfahren von Anspruch 19 oder 20.

22. Membranpräparat, isoliert aus der Wirtszelle nach einem der Ansprüche 11 bis 18, wobei eine solche Wirtszelle einen Y5-Rezeptor nicht von Natur aus exprimiert.

23. Antikörper, der zur Bindung an den Rezeptor nach Anspruch 21 befähigt ist.

24. Antikörper, der zur kompetitiven Hemmung der Bindung des Antikörpers nach Anspruch 23 an einen Y5-Rezeptor befähigt ist.

25. Antikörper nach Anspruch 23 oder 24, der ein monoclonaler Antikörper ist.

26. Monoclonaler Antikörper nach Anspruch 25, der gegen ein Epitop eines Y5-Rezeptors gerichtet ist, das auf der Oberfläche einer Y5-Rezeptor exprimierenden Zelle vorhanden ist.

27. Arzneimittel, das den Antikörper nach einem der Ansprüche 25 bis 26 und einen pharmazeutischen verträglichen Träger umfasst.

28. Verfahren zur Bestimmung, ob eine chemische Verbindung an einen Y5-Rezeptor spezifisch bindet, welches umfasst: Inkontaktbringen von Wirtszellen nach Anspruch 11 oder des Membranpräparats nach Anspruch 22 mit der chemischen Verbindung unter Bedingungen, die für die Bindung geeignet sind, und Nachweisen der spezifischen Bindung der chemischen Verbindung an den Y5-Rezeptor.

29. Verfahren, das kompetitive Bindung einschließt, zur Identifizierung einer chemischen Verbindung, die an einen Y5-Rezeptor spezifisch bindet, welches umfasst: getrenntes Inkontaktbringen von Wirtszellen nach Anspruch 11 oder des Membranpräparats nach Anspruch 22 mit sowohl der chemischen Verbindung als auch einer zweiten chemischen Verbindung, von der bekannt ist, dass sie an den Y5-Rezeptor bindet, und nur mit der zweiten chemischen Verbindung unter Bedingungen, die für die Bindung der Verbindungen geeignet sind; und Nachweisen der spezifischen Bindung der chemischen Verbindung an den Y5-Rezeptor; wobei ein Rückgang der Bindung der zweiten chemischen Verbindung an den Y5-Rezeptor in Anwesenheit der chemischen Verbindung anzeigt, dass

die chemische Verbindung an den Y5-Rezeptor bindet.

30. Verfahren, das kompetitive Bindung einschließt, zur Identifizierung einer chemischen Verbindung, die an einen Y5-Rezeptor spezifisch bindet, welches umfasst: getrenntes Inkontaktbringen von Wirtszellen nach Anspruch 11 oder des Membranpräparats nach Anspruch 22 mit sowohl einer chemischen Verbindung, von der bekannt ist, dass sie an den Y5-Rezeptor spezifisch bindet, als auch einer Vielzahl von chemischen Verbindungen, von denen nicht bekannt ist, ob sie an den Y5-Rezeptor spezifisch binden, und nur mit der chemischen Verbindung, von der bekannt ist, dass sie an den Y5-Rezeptor bindet, unter Bedingungen, die für die Bindung der Verbindungen geeignet sind; Nachweisen der spezifischen Bindung der Vielzahl von chemischen Verbindungen; wobei ein Rückgang der Bindung der chemischen Verbindung, die bekanntermaßen an den Y5-Rezeptor bindet, in Anwesenheit der Vielzahl von chemischen Verbindungen anzeigt, dass mindestens eine chemische Verbindung, die in der Vielzahl der chemischen Verbindungen enthalten ist, an den Y5-Rezeptor bindet; und getrenntes Nachweisen der Bindung jeder chemischen Verbindung, die in der Vielzahl der Verbindungen eingeschlossen ist, an den Y5-Rezeptor.

31. Verfahren für den Nachweis, ob eine chemische Verbindung ein Y5-Rezeptor-Agonist ist, welches umfasst: Inkontaktbringen von Wirtszellen nach Anspruch 11 oder des Membranpräparats nach Anspruch 22 mit der chemischen Verbindung unter Bedingungen, die die Aktivierung des Y5-Rezeptors erlauben, und Nachweisen eines Anstiegs der Y5-Rezeptor-Aktivität, um dadurch zu bestimmen, ob die chemische Verbindung ein Y5-Rezeptor-Agonist ist.

32. Verfahren zur Bestimmung, ob eine chemische Verbindung an einen Y5-Rezeptor spezifisch bindet und ihn aktiviert, welches umfasst: Inkontaktbringen von Wirtszellen nach Anspruch 11 oder des Membranpräparats nach Anspruch 22 mit der chemischen Verbindung unter Bedingungen, die für die Aktivierung des Y5-Rezeptors geeignet sind, und Messen einer Second-Messenger-Antwort in Anwesenheit und Abwesenheit der chemischen Verbindung, wobei eine Änderung der Second-Messenger-Antwort in Anwesenheit der chemischen Verbindung anzeigt, dass die chemische Verbindung den Y5-Rezeptor aktiviert.

33. Verfahren zur Bestimmung, ob eine chemische Verbindung an einen Y5-Rezeptor spezifisch bindet und ihn aktiviert, welches umfasst: Inkontaktbringen von Wirtszellen nach Anspruch 11 oder des Membranpräparats nach Anspruch 22 mit einer Vielzahl von chemischen Verbindungen, von denen nicht bekannt ist, ob sie an den Y5-Rezeptor binden und ihn aktivieren, unter Bedingungen, die für die Aktivierung des Y5-Rezeptors geeignet sind; Messen einer Second-Messenger-Antwort in Anwesenheit und Abwesenheit der Vielzahl von chemischen Verbindungen; wobei eine Änderung der Second-Messenger-Antwort in Anwesenheit der Vielzahl von chemischen Verbindungen anzeigt, dass mindestens eine chemische Verbindung aus der Vielzahl von chemischen Verbindungen den Y5-Rezeptor aktiviert; und getrenntes Bestimmen, ob jede Verbindung, die in der Vielzahl von Verbindungen eingeschlossen ist, an den Y5-Rezeptor bindet und ihn aktiviert.

34. Verfahren nach Anspruch 32 oder 33, worin die Second-Messenger-Antwort Adenylatcyclase-Aktivität umfasst und die Änderung der Second-Messenger-Antwort ein Rückgang der Adenylatcyclase-Aktivität ist.

35. Verfahren nach Anspruch 32 oder 33, worin die Second-Messenger-Antwort die intrazelluläre Calciumkonzentration umfasst und die Änderung der Second-Messenger-Antwort ein Anstieg der intrazellulären Calciumkonzentration ist.

36. Verfahren zur Bestimmung, ob eine chemische Verbindung ein Y5-Rezeptor-Antagonist ist, welches umfasst: Inkontaktbringen von Wirtszellen nach Anspruch 11 oder des Membranpräparats nach Anspruch 22 mit der chemischen Verbindung in Anwesenheit eines bekannten Y5-Rezeptor-Agonisten unter Bedingungen, die die Aktivierung des Y5-Rezeptors erlauben, und Nachweisen eines Rückgangs der Y5-Rezeptor-Aktivität, um dadurch zu bestimmen, ob die chemische Verbindung ein Y5-Rezeptor-Antagonist ist.

37. Verfahren zur Bestimmung, ob eine chemische Verbindung an einen Y5-Rezeptor spezifisch bindet und seine Aktivierung hemmt, welches umfasst: getrenntes Inkontaktbringen von Wirtszellen nach Anspruch 11 oder des Membranpräparats nach Anspruch 22 mit sowohl der chemischen Verbindung als auch einer zweiten chemischen Verbindung, von der bekannt ist, dass sie den Y5-Rezeptor aktiviert, und nur mit der zweiten chemischen Verbindung unter Bedingungen, die für die Aktivierung des Y5-Rezeptors geeignet sind; und Messen einer Second-Messenger-Antwort in Anwesenheit von nur der zweiten chemischen Verbindung und in Anwesenheit von sowohl der zweiten chemischen Verbindung als auch der chemischen Verbindung, wobei eine kleinere Änderung der Second-Messenger-Antwort in Anwesenheit von sowohl der chemischen Verbindung als auch der zweiten chemi-

schen Verbindung als in Anwesenheit von nur der zweiten chemischen Verbindung anzeigt, dass die chemische Verbindung die Aktivierung des Y5-Rezeptors hemmt.

38. Verfahren zur Bestimmung, ob eine chemische Verbindung an einen Y5-Rezeptor spezifisch bindet und seine Aktivierung hemmt, welches umfasst: getrenntes Inkontaktbringen von Wirtszellen nach Anspruch 11 oder des Membranpräparats nach Anspruch 22 mit sowohl einer chemischen Verbindung, von der bekannt ist, dass sie die Y5-Rezeptor aktiviert, als auch einer Vielzahl von chemischen Verbindungen, von denen nicht bekannt ist, ob sie die Aktivierung des Y5-Rezeptors hemmen, und mit nur der chemischen Verbindung, von der bekannt ist, dass sie den Y5-Rezeptor aktiviert, unter Bedingungen, die für die Aktivierung des Y5-Rezeptors geeignet sind; und Messen einer Second-Messenger-Antwort in Anwesenheit von nur der chemischen Verbindung, von der bekannt ist, dass sie den Y5-Rezeptor aktiviert, und in Anwesenheit von sowohl der chemischen Verbindung, von der bekannt ist, dass sie den Y5-Rezeptor aktiviert, als auch der Vielzahl von chemischen Verbindungen; wobei eine kleinere Änderung der Second-Messenger-Antwort in Anwesenheit von sowohl der chemischen Verbindung, von der bekannt ist, dass sie den Y5-Rezeptor aktiviert, als auch der Vielzahl von chemischen Verbindungen als bei Anwesenheit von nur der chemischen Verbindung, von der bekannt ist, dass sie den Y5-Rezeptor aktiviert, anzeigt, dass mindestens eine chemische Verbindung, die in der Vielzahl von chemischen Verbindungen eingeschlossen ist, die Aktivierung des Y5-Rezeptors hemmt; und getrenntes Bestimmen ob jede Verbindung, die in der Vielzahl der chemischen Verbindungen eingeschlossen ist, an den Y5-Rezeptor spezifisch bindet und seine Aktivierung hemmt.

39. Verfahren nach Anspruch 37 oder 38, worin die Second-Messenger-Antwort Adenylatcyclase-Aktivität umfasst und die Änderung der Second-Messenger-Antwort ein kleinerer Rückgang des Adenylatcyclase-Aktivitätsniveaus in Anwesenheit von sowohl der chemischen Verbindung als auch der zweiten chemischen Verbindung oder der chemischen Verbindung, von der bekannt ist, dass sie den Y5-Rezeptor aktiviert, und der Vielzahl von chemischen Verbindungen ist als in Anwesenheit von nur der zweiten chemischen Verbindung oder der chemischen Verbindung, von der bekannt ist, dass sie den Y5-Rezeptor aktiviert.

40. Verfahren nach Anspruch 37 oder 38, worin die Second-Messenger-Antwort die intrazelluläre Calciumkonzentration umfasst und die Änderung der Second-Messenger-Antwort ein kleinerer Anstieg der intrazellulären Calciumkonzentration in Anwesenheit von sowohl der chemischen Verbindung als auch der zweiten chemischen Verbindung oder der chemischen Verbindung, von der bekannt ist, dass sie den Y5-Rezeptor aktiviert, und der Vielzahl von chemischen Verbindungen ist als in Anwesenheit von nur der zweiten chemischen Verbindung oder der chemischen Verbindung, von der bekannt ist, dass sie den Y5-Rezeptor aktiviert.

41. Verfahren für den Nachweis der Anwesenheit eines menschlichen Y5-Rezeptors auf der Oberfläche einer Zelle in vitro, welches umfasst: Inkontaktbringen der Zelle mit dem Antikörper nach einem der Ansprüche 23 bis 26 unter Bedingungen, die die Bindung des Antikörpers an den Rezeptor erlauben; Nachweisen der Anwesenheit des Antikörpers, der an die Zelle gebunden ist, und dadurch Nachweisen der Anwesenheit eines menschlichen Y5-Rezeptors auf der Oberfläche der Zelle.

42. Verfahren zur Herstellung einer Zusammensetzung, das umfasst:

    Bestimmen, ob eine chemische Verbindung ein Y5-Rezeptor-Agonist ist unter Verwendung des Verfahrens nach Anspruch 31; Abtrennen der chemischen Verbindung, die als Y5-Rezeptor-Agonist bestimmt wurde, von den Wirtszellen oder dem Membranpräparat; und Einbringen der chemischen Verbindung in einen Träger.

43. Verfahren zur Herstellung einer Zusammensetzung, das umfasst:

    Bestimmen, ob eine chemische Verbindung ein Y5-Rezeptor-Antagonist ist unter Verwendung des Verfahrens nach Anspruch 36; Abtrennen der chemischen Verbindung, die als Y5-Rezeptor Antagonist bestimmt wurde, von den Wirtszellen oder dem Membranpräparat; und Einbringen der chemischen Verbindung in einen Träger.

Revendications

1. Acide nucléique codant pour un récepteur Y5 mammifère, ledit acide nucléique contenant :

    a) une séquence de nucléotides codant pour la séquence d'aminoacides représentée sur la figure 6 ; ou

b) une séquence de nucléotides codant pour la séquence d'aminoacides représentée sur la figure 4 ; ou
c) une séquence de nucléotides codant pour la séquence d'aminoacides représentée sur la figure 15 ; ou
d) une séquence de nucléotides qui code pour un récepteur Y5 dans un mammifère autre qu'un être humain, un rat, ou un canidé et qui est hybridé dans des conditions convenables à une quelconque des séquences (a) à (c) ;

dans lequel le récepteur est **caractérisé par** (1) un profil pharmacologique caractéristique du récepteur Y5 humain représenté dans le tableau 6 ou dans le tableau 7 ; ou (2) un profil pharmacologique caractéristique du récepteur Y5 de rat représenté dans le tableau 4 ou le tableau 5.

2. Acide nucléique selon la revendication 1, qui est de l'ADN ou de l'ARN, dans lequel s'il c'est de l'ARN, l'ARN est de préférence l'ARN messager.

3. Acide nucléique selon la revendication 2, qui est l'ADN complémentaire ou l'ADN du génome.

4. Acide nucléique selon la revendication 1, dans lequel l'acide nucléique code pour un récepteur Y5 **caractérisé par** une séquence d'aminoacides dans chacune des régions transmembranaires I-VII qui est identique à la séquence d'aminoacides dans la région transmembranaire correspondante du récepteur Y5 humain représentée sur la figure 8.

5. Protéine de récepteur Y5 purifiée codée par la molécule d'acide nucléique selon l'une quelconque des revendication 1 à 4.

6. Vecteur comprenant l'acide nucléique selon l'une quelconque des revendication 1 à 4.

7. Vecteur selon la revendication 6 adapté pour une expression dans un cellule hôte qui comprend les éléments de régulation nécessaires à une expression de l'acide nucléique dans la cellule hôte liée au plan opératoire à l'acide nucléique codant pour un récepteur Y5 de façon à permettre son expression.

8. Vecteur selon la revendication 7, dans lequel la cellule hôte est une cellule bactérienne, de levure, d'insecte ou mammifère.

9. Vecteur selon la revendication 8 qui est un baculovirus ou un plasmide.

10. Plasmide selon la revendication 9 qui est pcEXV-hY5 (N° d'accession ATCC 75943) ou pcEXV-rY5 (N° d'accession ATCC 75944).

11. Cellule hôte comprenant le vecteur selon l'une quelconque des revendications 6 à 10.

12. Cellule hôte selon la revendication 11, qui est une cellule bactérienne, de levure, d'insecte ou mammifère.

13. Cellule hôte selon la revendication 11 ou 12, qui est d'origine non neuronale.

14. Cellule hôte selon la revendication 13, qui est une cellule COS-7, une cellule embryonnaire de rein humaine 293, une cellule NIH-3T3 ou une cellule LM(tk).

15. Cellule embryonnaire de rein humaine 293 selon la revendication 14, qui est 293-rY5-14 (N° d'accession ATCC CRL 11757).

16. Cellule NIH-3T3 selon la revendication 14, qui est N-hY5-8 (N° d'accession ATCC CRL 11994).

17. Cellule LM(tk) selon la revendication 14, qui est L-hY5-7 (N° d'accession ATCC CRL 11995).

18. Cellule hôte selon la revendication 12, dans laquelle la cellule d'insecte est une cellule 8f9 ou une cellule Sf21.

19. Procédé de préparation du récepteur Y5 humain, de rat ou canin purifié selon la revendication 5, qui comprend :

a. la construction d'un vecteur destiné à une expression dans une cellule qui comprend les éléments de ré-

gulation nécessaires à une expression d'un acide nucléique dans la cellule liée au plan opératoire à l'acide nucléique codant pour un récepteur Y5 humain, de rat ou canin de façon à permettre son expression, dans laquelle la cellule est choisie dans le groupe constitué par les cellules bactériennes, les cellules de levure, les cellules d'insecte et les cellules mammifères ;

b. l'insertion du vecteur de l'étape a dans une cellule hôte convenable ;

c. l'incubation des cellules de l'étape b dans des conditions permettant l'expression du récepteur Y5 humain, de rat ou canin ;

d. la récupération du récepteur ainsi produit ; et

e. la purification du récepteur ainsi récupéré, afin de préparer un récepteur Y5 humain, de rat ou canin.

20. Procédé de préparation d'un récepteur Y5 purifié qui comprend :

(a) le fait de mettre la cellule hôte selon l'une quelconque des revendications 11 à 18 dans des conditions convenables permettant la production du récepteur Y5 ;

(b) la récupération du récepteur ainsi produit par la cellule hôte ; et

(c) la purification du récepteur ainsi récupéré.

21. Récepteur Y5 purifié préparé par le procédé selon la revendication 19 ou 20.

22. Préparation de membrane isolée de la cellule hôte selon l'une quelconque des revendications 11 à 18, cellule hôte qui n'exprime pas naturellement un récepteur Y5.

23. Anticorps capable de se lier au récepteur selon la revendication 21.

24. Anticorps capable d'inhiber de façon concurrente la liaison à un récepteur Y5 de l'anticorps selon la revendication 23.

25. Anticorps selon la revendication 23 ou 24, qui est un anticorps monoclonal.

26. Anticorps monoclonal selon la revendication 25, qui est dirigé contre un épitope d'un récepteur Y5 présent à la surface d'une cellule exprimant un récepteur Y5.

27. Composition pharmaceutique qui comprend l'anticorps selon l'une quelconque des revendications 25 et 26 et un véhicule acceptable au plan pharmaceutique.

28. Procédé pour déterminer si un composé chimique se lie spécifiquement à un récepteur Y5, qui comprend la mise en contact de cellules hôtes selon la revendication 11, ou la préparation de membrane selon la revendication 22, avec le composé chimique dans des conditions convenant à une liaison, et la détection de la liaison spécifique du composé chimique au récepteur Y5.

29. Procédé mettant en jeu une liaison concurrente pour identifier un composé chimique qui se lie spécifiquement à un récepteur Y5, qui comprend de façon séparée la mise en contact de cellules hôtes selon la revendication 11, ou la préparation de membrane selon la revendication 22, à la fois avec le composé chimique et un second composé chimique connu pour se lier au récepteur Y5, et uniquement avec le second composé chimique, dans des conditions convenant à la liaison des composés, et la détection de la liaison spécifique du composé chimique au récepteur Y5, une diminution de la liaison du second composé chimique au récepteur Y5 en présence du composé chimique indiquant que le composé chimique se lie au récepteur Y5.

30. Procédé mettant en jeu une liaison concurrente pour identifier un composé chimique qui se lie spécifiquement à un récepteur Y5, qui comprend de façon séparée la mise en contact de cellules hôtes selon la revendication 11, ou la préparation de membrane selon la revendication 22, à la fois avec un composé chimique connu pour se lier spécifiquement au récepteur Y5 et avec une pluralité de composés chimiques non connus pour se lier spécifiquement au récepteur Y5, et uniquement avec le composé chimique connu pour se lier au récepteur Y5, dans des conditions convenant à la liaison des composés, et la détection de la liaison spécifique de la pluralité de composés chimiques, une diminution de la liaison du composé chimique connu pour se lier au récepteur Y5 en présence de la pluralité de composés chimiques indiquant qu'au moins un composé chimique compris dans la pluralité de composés chimiques se lie au récepteur Y5, et la détection de façon séparée de la liaison de chaque composé chimique compris dans la pluralité de composés au récepteur Y5.

31. Procédé pour déterminer si un composé chimique est un agoniste du récepteur Y5, qui comprend la mise en contact de cellules hôtes selon la revendication 11, ou la préparation de membrane selon la revendication 22, avec le composé chimique dans des conditions permettant une activation du récepteur Y5, et la détection d'une augmentation de l'activité du récepteur Y5, de façon à déterminer si le composé chimique est un agoniste du récepteur Y5.

32. Procédé pour déterminer si un composé chimique se lie spécifiquement à et active un récepteur Y5, qui comprend la mise en contact de cellules hôtes selon la revendication 11, ou la préparation de membrane selon la revendication 22, avec le composé chimique dans des conditions permettant une activation du récepteur Y5, et la mesure d'une seconde réponse messagère en présence et en l'absence du composé chimique, une variation de la seconde réponse messagère en présence du composé chimique indiquant que le composé chimique active le récepteur Y5.

33. Procédé pour déterminer si un composé chimique se lie spécifiquement à et active un récepteur Y5, qui comprend la mise en contact de cellules hôtes selon la revendication 11, ou la préparation de membrane selon la revendication 22, avec une pluralité de composés chimiques non connus pour se lier à et activer le récepteur Y5, dans des conditions permettant une activation du récepteur Y5, la mesure d'une seconde réponse messagère en présence et en l'absence de la pluralité de composés chimiques, une variation de la seconde réponse messagère en présence de la pluralité de composés chimiques indiquant qu'au moins un composé chimique dans la pluralité de composés chimiques active le récepteur Y5, et le fait de déterminer de façon séparée si chaque composé compris dans la pluralité de composés se lie à et active le récepteur Y5.

34. Procédé selon la revendication 32 ou 33, dans lequel la seconde réponse messagère comprend l'activité de l'adénylate cyclase et la variation de la seconde réponse messagère est une diminution de l'activité de l'adénylate cyclase.

35. Procédé selon la revendication 32 ou 33, dans lequel la seconde réponse messagère comprend la concentration en calcium intracellulaire et la variation de la seconde réponse messagère est une augmentation de la concentration en calcium intracellulaire.

36. Procédé pour déterminer si un composé chimique est un antagoniste du récepteur Y5, qui comprend la mise en contact de cellules hôtes selon la revendication 11, ou la préparation de membrane selon la revendication 22, avec le composé chimique en présence d'un agoniste du récepteur Y5 connu, dans des conditions permettant une activation du récepteur Y5, et la détection d'une diminution de l'activité du récepteur Y5, de façon à déterminer si le composé chimique est un antagoniste du récepteur Y5.

37. Procédé pour déterminer si un composé chimique se lie spécifiquement à un, ou inhibe l'activation d'un, récepteur Y5, qui comprend de façon séparée la mise en contact de cellules hôtes selon la revendication 11, ou la préparation de membrane selon la revendication 22, à la fois avec le composé chimique et un second composé chimique connu pour activer le récepteur Y5, et uniquement avec le second composé chimique, dans des conditions convenant à l'activation du récepteur Y5, et la mesure d'une seconde réponse messagère en présence uniquement du second composé chimique et en présence à la fois du second composé chimique et du composé chimique, une variation plus faible de la seconde réponse messagère en présente à la fois du composé chimique et du second composé chimique qu'en présence uniquement du second composé chimique indiquant que le composé chimique inhibe l'activation du récepteur Y5.

38. Procédé pour déterminer si un composé chimique se lie spécifiquement à un, ou inhibe l'activation d'un, récepteur Y5, qui comprend de façon séparée la mise en contact de cellules hôtes selon la revendication 11, ou la préparation de membrane selon la revendication 22, à la fois avec un composé chimique connu pour activer le récepteur Y5 et avec une pluralité de composés chimiques non connus pour inhiber l'activation du récepteur Y5, et uniquement avec le composé chimique connu pour activer le récepteur Y5, dans des conditions convenant à l'activation du récepteur Y5, et la mesure d'une seconde réponse messagère en présence uniquement du composé chimique connu pour activer le récepteur Y5 et en présence à la fois du composé chimique connu pour activer le récepteur Y5 et de la pluralité de composés chimiques, une variation plus faible de la seconde réponse messagère en présence à la fois du composé chimique connu pour activer le récepteur Y5 et de la pluralité de composés chimiques qu'en présence uniquement du composé chimique connu pour activer le récepteur Y5 indiquant qu'au moins un composé chimique compris dans la pluralité de composés chimiques inhibe l'activation du récepteur Y5 et le fait de déterminer de façon séparée si chaque composé compris dans la pluralité de composés chimiques se lie à et inhibe l'activation du récepteur Y5.

39. Procédé selon la revendication 37 ou 38, dans lequel la seconde réponse messagère comprend l'activité de l'adénylate cyclase et la variation de la seconde réponse messagère est une diminution plus faible du niveau d'activité de l'adénylate cyclase en présence à la fois du composé chimique et du second composé chimique, ou du composé chimique connu pour activer le récepteur Y5 et de la pluralité de composés chimiques, qu'en présence uniquement du second composé chimique, ou du composé chimique connu pour activer le récepteur Y5.

40. Procédé selon la revendication 37 ou 38, dans lequel la seconde réponse messagère comprend une concentration en calcium intracellulaire et la variation de la seconde réponse messagère est une diminution plus faible de la concentration en calcium intracellulaire en présence à la fois du composé chimique et du second composé chimique, ou du composé chimique connu pour activer le récepteur Y5 et de la pluralité de composés chimiques, qu'en présence uniquement du second composé chimique, ou du composé chimique connu pour activer le récepteur Y5.

41. Procédé de détection de la présence d'un récepteur Y5 humain sur la surface d'une cellule in vitro qui comprend la mise en contact de la cellule avec un anticorps selon l'une quelconque des revendications 23 à 26 dans des conditions permettant la liaison de l'anticorps au récepteur, la détection de la présence de l'anticorps lié à la cellule, et de ce fait la détection de la présence d'un récepteur Y5 humain sur la surface de la cellule.

42. Procédé de préparation d'une composition qui comprend le fait de déterminer si un composé chimique est un agoniste du récepteur Y5 à l'aide du procédé de la revendication 31, la séparation du composé chimique qui a été ainsi déterminé comme étant un agoniste du récepteur Y5 des cellules hôtes ou de la préparation de membrane, et la mise en place du composé chimique dans un véhicule.

43. Procédé de préparation d'une composition qui comprend le fait de déterminer si un composé chimique est un antagoniste du récepteur Y5 à l'aide du procédé de la revendication 36, la séparation du composé chimique qui a été ainsi déterminé comme étant un antagoniste du récepteur Y5 des cellules hôtes ou de la préparation de membrane, et la mise en place du composé chimique dans un véhicule.

FIGURE 1

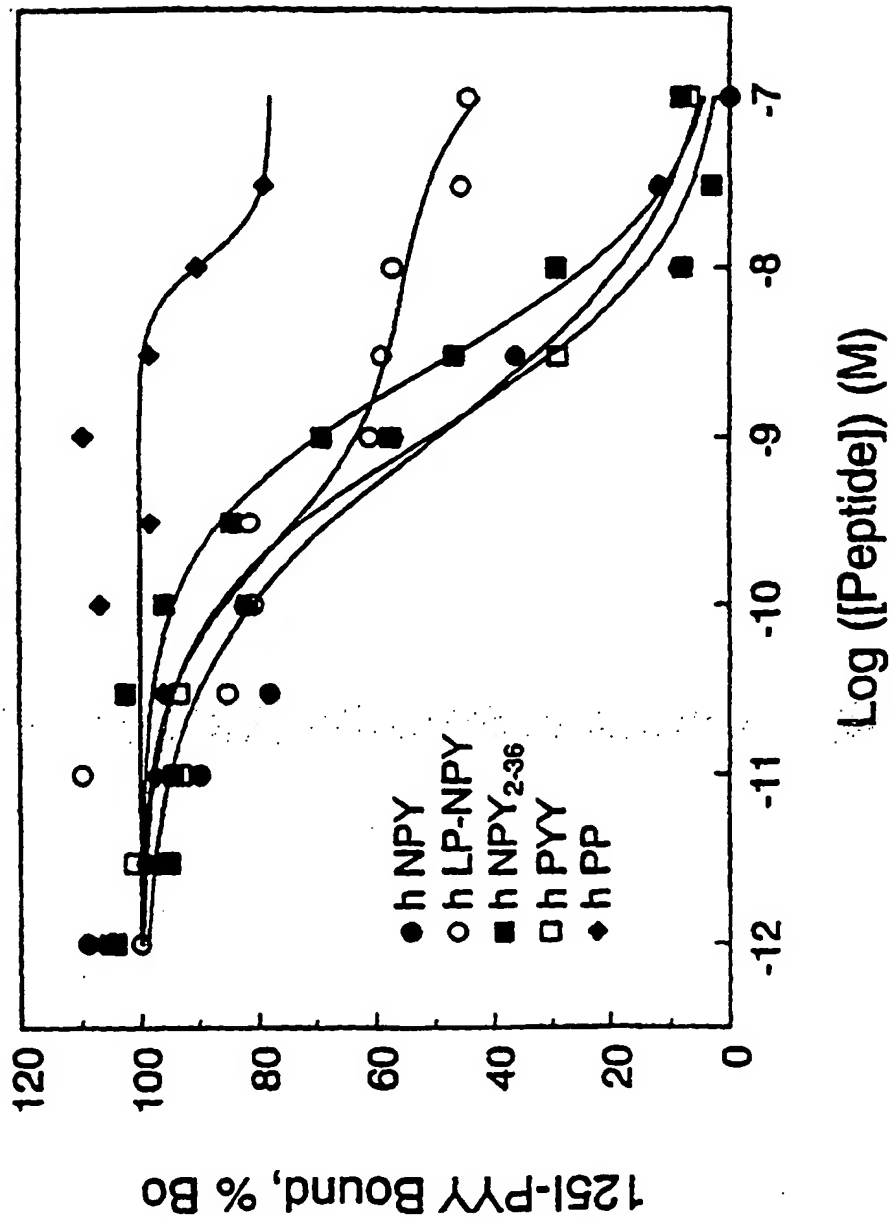

125I-PYY Bound, % Bo
120
100
80
60
40
20
0
-12
-11
-10
-9
-8
-7
Log ([Peptide]) (M)
h NPY
h LP-NPY
h NPY2-36
h PYY
h PP

FIGURE 2

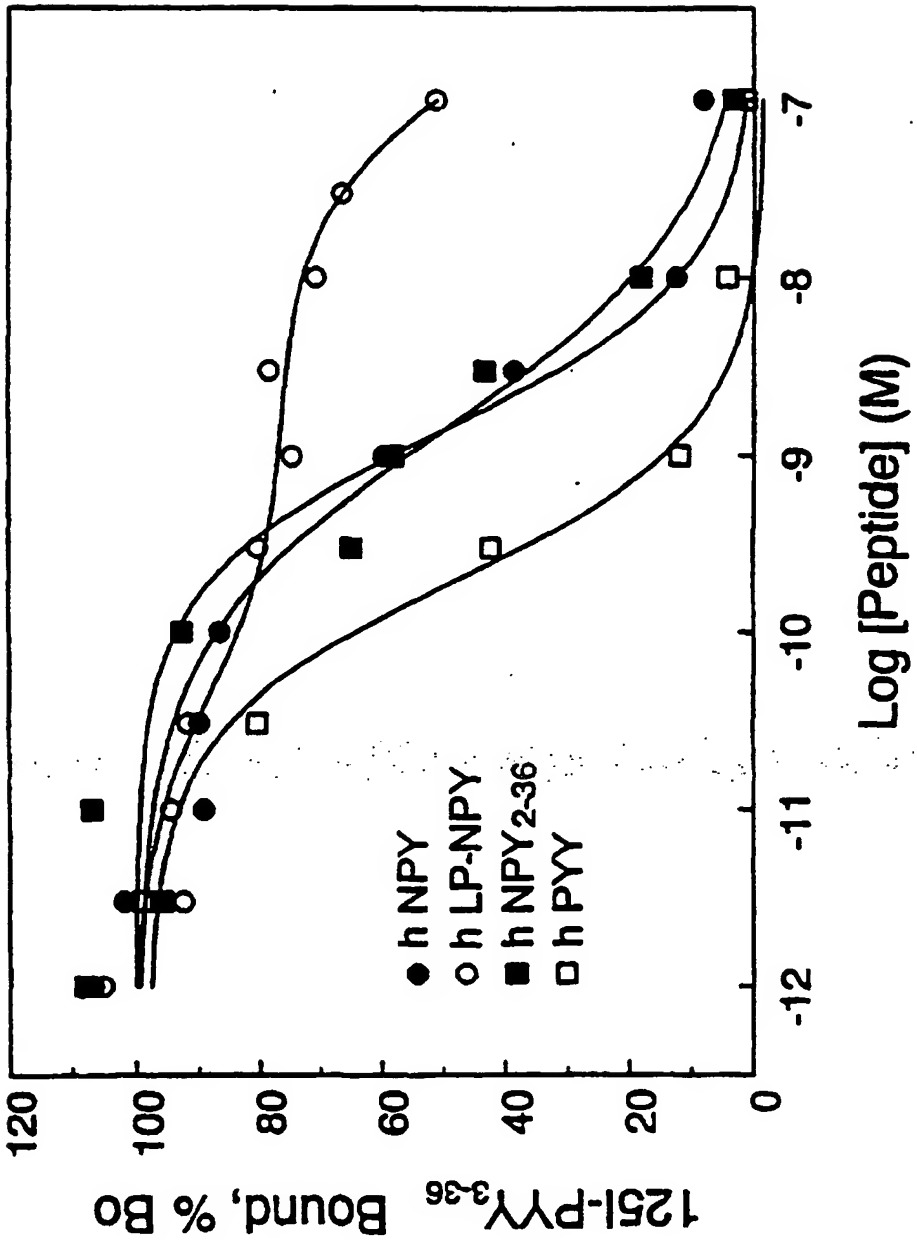
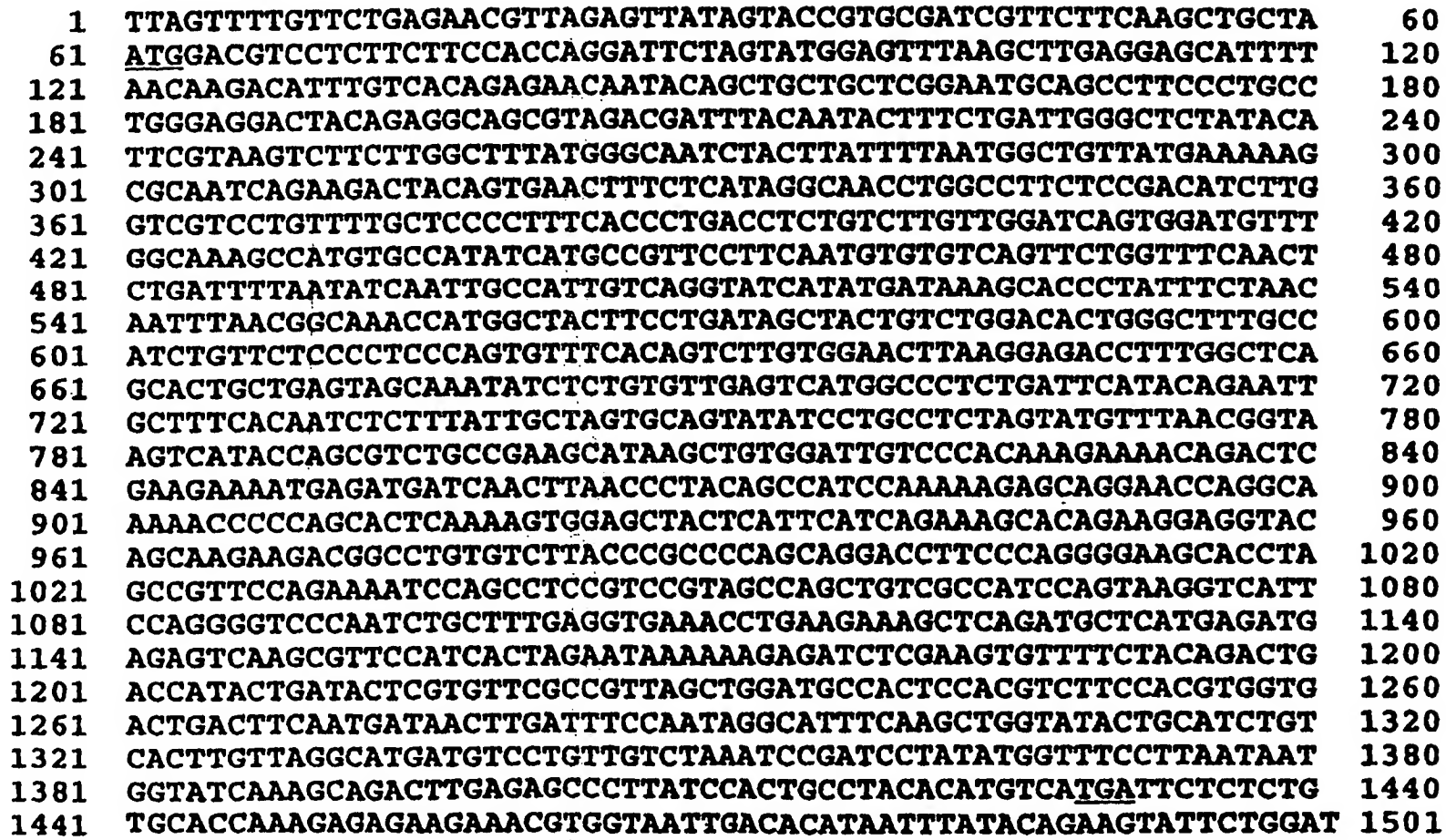

125I-PYY3-36 Bound, % Bo
120
100
80
60
40
20
0
-12
-11
-10
-9
-8
-7
Log [Peptide] (M)
h NPY
h LP-NPY
h NPY2-36
h PYY

FIGURE 3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61 ATGGACGTCCTCTTCTTCCACCAGGATTCTAGTATGGAGTTTAAGCTTGAGGAGCATTTT 120
121 AACAAGACATTTGTCACAGAGAACAATACAGCTGCTGCTCGGAATGCAGCCTTCCCTGCC 180
181 TGGGAGGACTACAGAGGCAGCGTAGACGATTTACAATACTTTCTGATTGGGCTCTATACA 240
241 TTCGTAAGTCTTCTTGGCTTTATGGGCAATCTACTTATTTTAATGGCTGTTATGAAAAAG 300
301 CGCAATCAGAAGACTACAGTGAACTTTCTCATAGGCAACCTGGCCTTCTCCGACATCTTG 360
361 GTCGTCCTGTTTTGCTCCCCTTTCACCCTGACCTCTGTCTTGTTGGATCAGTGGATGTTT 420
421 GGCAAAGCCATGTGCCATATCATGCCGTTCCTTCAATGTGTGTCAGTTCTGGTTTCAACT 480
481 CTGATTTTAATATCAATTGCCATTGTCAGGTATCATATGATAAAGCACCCTATTTCTAAC 540
541 AATTTAACGGCAAACCATGGCTACTTCCTGATAGCTACTGTCTGGACACTGGGCTTTGCC 600
601 ATCTGTTCTCCCCTCCCAGTGTTTCACAGTCTTGTGGAACTTAAGGAGACCTTTGGCTCA 660
661 GCACTGCTGAGTAGCAAATATCTCTGTGTTGAGTCATGGCCCTCTGATTCATACAGAATT 720
721 GCTTTCACAATCTCTTTATTGCTAGTGCAGTATATCCTGCCTCTAGTATGTTTAACGGTA 780
781 AGTCATACCAGCGTCTGCCGAAGCATAAGCTGTGGATTGTCCCACAAAGAAAACAGACTC 840
841 GAAGAAAATGAGATGATCAACTTAACCCTACAGCCATCCAAAAAGAGCAGGAACCAGGCA 900
901 AAAACCCCCAGCACTCAAAAGTGGAGCTACTCATTCATCAGAAAGCACAGAAGGAGGTAC 960
961 AGCAAGAAGACGGCCTGTGTCTTACCCGCCCCAGCAGGACCTTCCCAGGGGAAGCACCTA 1020
1021 GCCGTTCCAGAAAATCCAGCCTCCGTCCGTAGCCAGCTGTCGCCATCCAGTAAGGTCATT 1080
1081 CCAGGGGTCCCAATCTGCTTTGAGGTGAAACCTGAAGAAAGCTCAGATGCTCATGAGATG 1140
1141 AGAGTCAAGCGTTCCATCACTAGAATAAAAAAGAGATCTCGAAGTGTTTTCTACAGACTG 1200
1201 ACCATACTGATACTCGTGTTCGCCGTTAGCTGGATGCCACTCCACGTCTTCCACGTGGTG 1260
1261 ACTGACTTCAATGATAACTTGATTTCCAATAGGCATTTCAAGCTGGTATACTGCATCTGT 1320
1321 CACTTGTTAGGCATGATGTCCTGTTGTCTAAATCCGATCCTATATGGTTTCCTTAATAAT 1380
1381 GGTATCAAAGCAGACTTGAGAGCCCTTATCCACTGCCTACACATGTCATGATTCTCTCTG 1440
1441 TGCACCAAAGAGAGAAGAAACGTGGTAATTGACACATAATTTATACAGAAGTATTCTGGAT 1501

FIGURE 4

1	M	D	V	L	F	F	H	Q	D	S	S	M	E	F	K	L	E	E	H	F	20
21	N	K	T	F	V	T	E	N	N	T	A	A	A	R	N	A	A	F	P	A	40
41	W	E	D	Y	R	G	S	V	D	D	L	Q	Y	F	L	I	G	L	Y	T	60
61	F	V	S	L	L	G	F	M	G	N	L	L	I	L	M	A	V	M	K	K	80
81	R	N	Q	K	T	T	V	N	F	L	I	G	N	L	A	F	S	D	I	L	100
101	V	V	L	F	C	S	P	F	T	L	T	S	V	L	L	D	Q	W	M	F	120
121	G	K	A	M	C	H	I	M	P	F	L	Q	C	V	S	V	L	V	S	T	140
141	L	I	L	I	S	I	A	I	V	R	Y	H	M	I	K	H	P	I	S	N	160
161	N	L	T	A	N	H	G	Y	F	L	I	A	T	V	W	T	L	G	F	A	180
181	I	C	S	P	L	P	V	F	H	S	L	V	E	L	K	E	T	F	G	S	200
201	A	L	L	S	S	K	Y	L	C	V	E	S	W	P	S	D	S	Y	R	I	220
221	A	F	T	I	S	L	L	L	V	Q	Y	I	L	P	L	V	C	L	T	V	240
241	S	H	T	S	V	C	R	S	I	S	C	G	L	S	H	K	E	N	R	L	260
261	E	E	N	E	M	I	N	L	T	L	Q	P	S	K	K	S	R	N	Q	A	280
281	K	T	P	S	T	Q	K	W	S	Y	S	F	I	R	K	H	R	R	R	Y	300
301	S	K	K	T	A	C	V	L	P	A	P	A	G	P	S	Q	G	K	H	L	320
321	A	V	P	E	N	P	A	S	V	R	S	Q	L	S	P	S	S	K	V	I	340
341	P	G	V	P	I	C	F	E	V	K	P	E	E	S	S	D	A	H	E	M	360
361	R	V	K	R	S	I	T	R	I	K	K	R	S	R	S	V	F	Y	R	L	380
381	T	I	L	I	L	V	F	A	V	S	W	M	P	L	H	V	F	H	V	V	400
401	T	D	F	N	D	N	L	I	S	N	R	H	F	K	L	V	Y	C	I	C	420
421	H	L	L	G	M	M	S	C	C	L	N	P	I	L	Y	G	F	L	N	N	440
441	G	I	K	A	D	L	R	A	L	I	H	C	L	H	M	S					456

FIGURE 5

```
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  61  ATGTCTTTTTATTCCAAGCAGGACTATAATATGGATTTAGAGCTCGACGAGTATTATAAC  120
 121  AAGACACTTGCCACAGAGAATAATACTGCTGCCACTCGGAATTCTGATTTCCCAGTCTGG  180
 181  GATGACTATAAAAGCAGTGTAGATGACTTACAGTATTTTCTGATTGGGCTCTATACATTT  240
 241  GTAAGTCTTCTTGGCTTTATGGGGAATCTACTTATTTTAATGGCTCTCATGAAAAAGCGT  300
 301  AATCAGAAGACTACGGTAAACTTCCTCATAGGCAATCTGGCCTTTTCTGATATCTTGGTT  360
 361  GTGCTGTTTTGCTCACCTTTCACACTGACGTCTGTCTTGCTGGATCAGTGGATGTTTGGC  420
 421  AAAGTCATGTGCCATATTATGCCTTTTCTTCAATGTGTGTCAGTTTTGGTTTCAACTTTA  480
 481  ATTTTAATATCAATTGCCATTGTCAGGTATCATATGATAAAACATCCCATATCTAATAAT  540
 541  TTAACAGCAAACCATGGCTACTTTCTGATAGCTACTGTCTGGACACTAGGTTTTGCCATC  600
 601  TGTTCTCCCCTTCCAGTGTTTCACAGTCTTGTGGAACTTCAAGAAACATTTGGTTCAGCA  660
 661  TTGCTGAGCAGCAGGTATTTATGTGTTGAGTCATGGCCATCTGATTCATACAGAATTGCC  720
 721  TTTACTATCTCTTTATTGCTAGTTCAGTATATTCTGCCCTTAGTTTGTCTTACTGTAAGT  780
 781  CATACAAGTGTCTGCAGAAGTATAAGCTGTGGATTGTCCAACAAAGAAAACAGACTTGAA  840
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 961  AAGAAGACAGCATGTGTGTTACCTGCTCCAGAAAGACCTTCTCAAGAGAACCACTCCAGA 1020
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1081  CCAGGGGTCCCCACTTGCTTTGAGATAAAACCTGAAGAAAATTCAGATGTTCATGAATTG 1140
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1261  ACTGATTTTAATGACAATCTTATTTCAAATAGGCATTTCAAGTTGGTGTATTGCATTTGT 1320
1321  CATTTGTTGGGCATGATGTCCTGTTGTCTTAATCCAATTCTATATGGGTTTCTTAATAAT 1380
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1441  TTTACCAAGGAAAGAAC                                             1457
```

FIGURE 6

1	M	S	F	Y	S	K	Q	D	Y	N	M	D	L	E	L	D	E	Y	Y	N	20
21	K	T	L	A	T	E	N	N	T	A	A	T	R	N	S	D	F	P	V	W	40
41	D	D	Y	K	S	S	V	D	D	L	Q	Y	F	L	I	G	L	Y	T	F	60
61	V	S	L	L	G	F	M	G	N	L	L	I	L	M	A	L	M	K	K	R	80
81	N	Q	K	T	T	V	N	F	L	I	G	N	L	A	F	S	D	I	L	V	100
101	V	L	F	C	S	P	F	T	L	T	S	V	L	L	D	Q	W	M	F	G	120
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141	I	L	I	S	I	A	I	V	R	Y	H	M	I	K	H	P	I	S	N	N	160
161	L	T	A	N	H	G	Y	F	L	I	A	T	V	W	T	L	G	F	A	I	180
181	C	S	P	L	P	V	F	H	S	L	V	E	L	Q	E	T	F	G	S	A	200
201	L	L	S	S	R	Y	L	C	V	E	S	W	P	S	D	S	Y	R	I	A	220
221	F	T	I	S	L	L	L	V	Q	Y	I	L	P	L	V	C	L	T	V	S	240
241	H	T	S	V	C	R	S	I	S	C	G	L	S	N	K	E	N	R	L	E	260
261	E	N	E	M	I	N	L	T	L	H	P	S	K	K	S	G	P	Q	V	K	280
281	L	S	G	S	H	K	W	S	Y	S	F	I	K	K	H	R	R	R	Y	S	300
301	K	K	T	A	C	V	L	P	A	P	E	R	P	S	Q	E	N	H	S	R	320
321	I	L	P	E	N	F	G	S	V	R	S	Q	L	S	S	S	S	K	F	I	340
341	P	G	V	P	T	C	F	E	I	K	P	E	E	N	S	D	V	H	E	L	360
361	R	V	K	R	S	V	T	R	I	K	K	R	S	R	S	V	F	Y	R	L	380
381	T	I	L	I	L	V	F	A	V	S	W	M	P	L	H	L	F	H	V	V	400
401	T	D	F	N	D	N	L	I	S	N	R	H	F	K	L	V	Y	C	I	C	420
421	H	L	L	G	M	M	S	C	C	L	N	P	I	L	Y	G	F	L	N	N	440
441	G	I	K	A	D	L	V	S	L	I	H	C	L	H	M						455

FIGURE 7A

FIGURE 7A
FIGURE 7B
FIGURE 7C
FIGURE 7D
FIGURE 7E

```
  1 ATGGACGTCCTCTTCTTCC.ACCAGGATTCTAGTATGGAGTTTAAGCTTG   50
    |  |||  |  ||  ||||  |  |||||  |  ||  ||||||  ||  ||||  |
  1 ....ATGTCTTTTTATTCCAAGCAGGACTATAATATGGATTTAGAGCTCG   46

 51 AGGAGCATTTTAACAAGACATTTGTCACAGAGAACAATACAGCTGCTGCT  100
    |  |||  |||  ||||||||||  |||  ||||||||||  |||||  |||||   ||
 47 ACGAGTATTATAACAAGACACTTGCCACAGAGAATAATACTGCTGCCACT   96

101 CGGAATGCAGCCTTCCCTGCCTGGGAGGACTACAGAGGCAGCGTAGACGA  150
    ||||||  |  |  |||||  |  ||||||  |||||  |  |  ||||  |||||  ||
 97 CGGAATTCTGATTTCCCAGTCTGGGATGACTATAAAAGCAGTGTAGATGA  146

151 TTTACAATACTTTCTGATTGGGCTCTATACATTCGTAAGTCTTCTTGGCT  200
     |||||  ||  ||||||||||||||||||||||  ||||||||||||||
147 CTTACAGTATTTTCTGATTGGGCTCTATACATTTGTAAGTCTTCTTGGCT  196

201 TTATGGGCAATCTACTTATTTTAATGGCTGTTATGAAAAAGCGCAATCAG  250
    |||||||  ||||||||||||||||||||||  |  ||||||||||  ||||||
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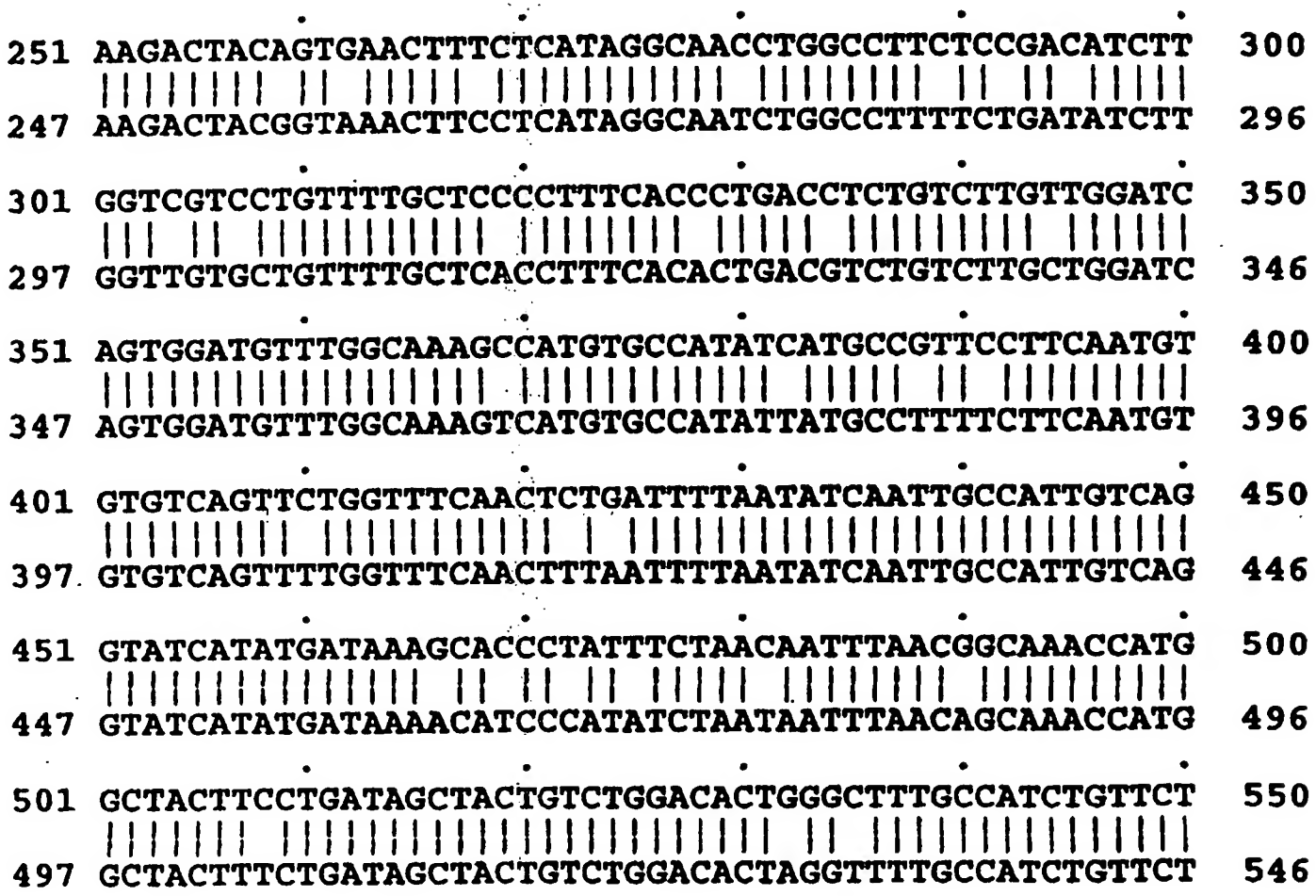
FIGURE 7B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351 AGTGGATGTTTGGCAAAGCCATGTGCCATATCATGCCGTTCCTTCAATGT 400
347 AGTGGATGTTTGGCAAAGTCATGTGCCATATTATGCCTTTTCTTCAATGT 396
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451 GTATCATATGATAAAGCACCCTATTTCTAACAATTTAACGGCAAACCATG 500
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501 GCTACTTCCTGATAGCTACTGTCTGGACACTGGGCTTTGCCATCTGTTCT 550
497 GCTACTTTCTGATAGCTACTGTCTGGACACTAGGTTTTGCCATCTGTTCT 546

FIGURE 7C

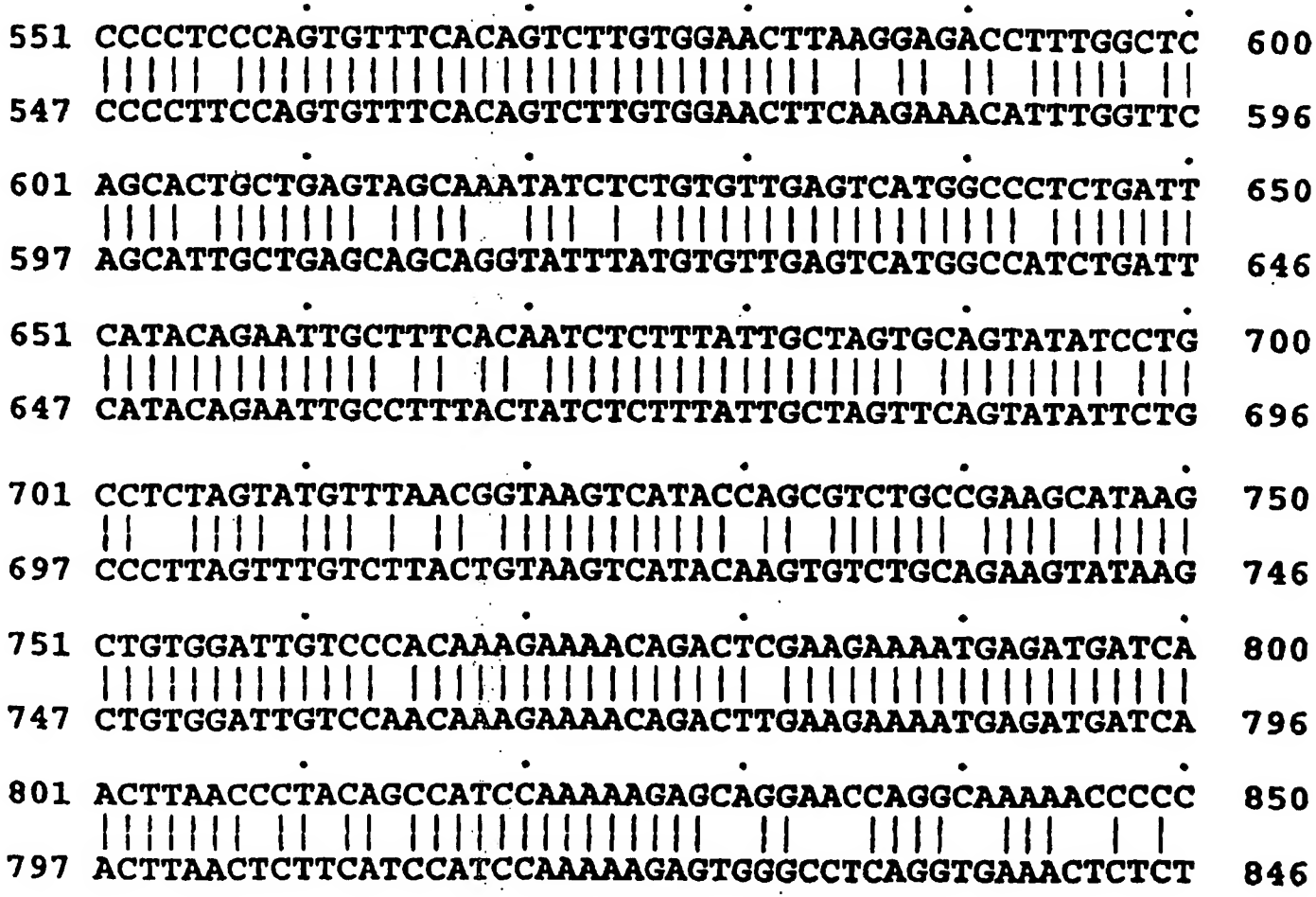
551 CCCCTCCCAGTGTTTCACAGTCTTGTGGAACTTAAGGAGACCTTTGGCTC 600
547 CCCCTTCCAGTGTTTCACAGTCTTGTGGAACTTCAAGAAACATTTGGTTC 596
601 AGCACTGCTGAGTAGCAAATATCTCTGTGTTGAGTCATGGCCCTCTGATT 650
597 AGCATTGCTGAGCAGCAGGTATTTATGTGTTGAGTCATGGCCATCTGATT 646
651 CATACAGAATTGCTTTCACAATCTCTTTATTGCTAGTGCAGTATATCCTG 700
647 CATACAGAATTGCCTTTACTATCTCTTTATTGCTAGTTCAGTATATTCTG 696
701 CCTCTAGTATGTTTAACGGTAAGTCATACCAGCGTCTGCCGAAGCATAAG 750
697 CCCTTAGTTTGTCTTACTGTAAGTCATACAAGTGTCTGCAGAAGTATAAG 746
751 CTGTGGATTGTCCCACAAAGAAAACAGACTCGAAGAAAATGAGATGATCA 800
747 CTGTGGATTGTCCAACAAAGAAAACAGACTTGAAGAAAATGAGATGATCA 796
801 ACTTAACCCTACAGCCATCCAAAAAGAGCAGGAACCAGGCAAAAACCCCC 850
797 ACTTAACTCTTCATCCATCCAAAAAGAGTGGGCCTCAGGTGAAACTCTCT 846

FIGURE 7D

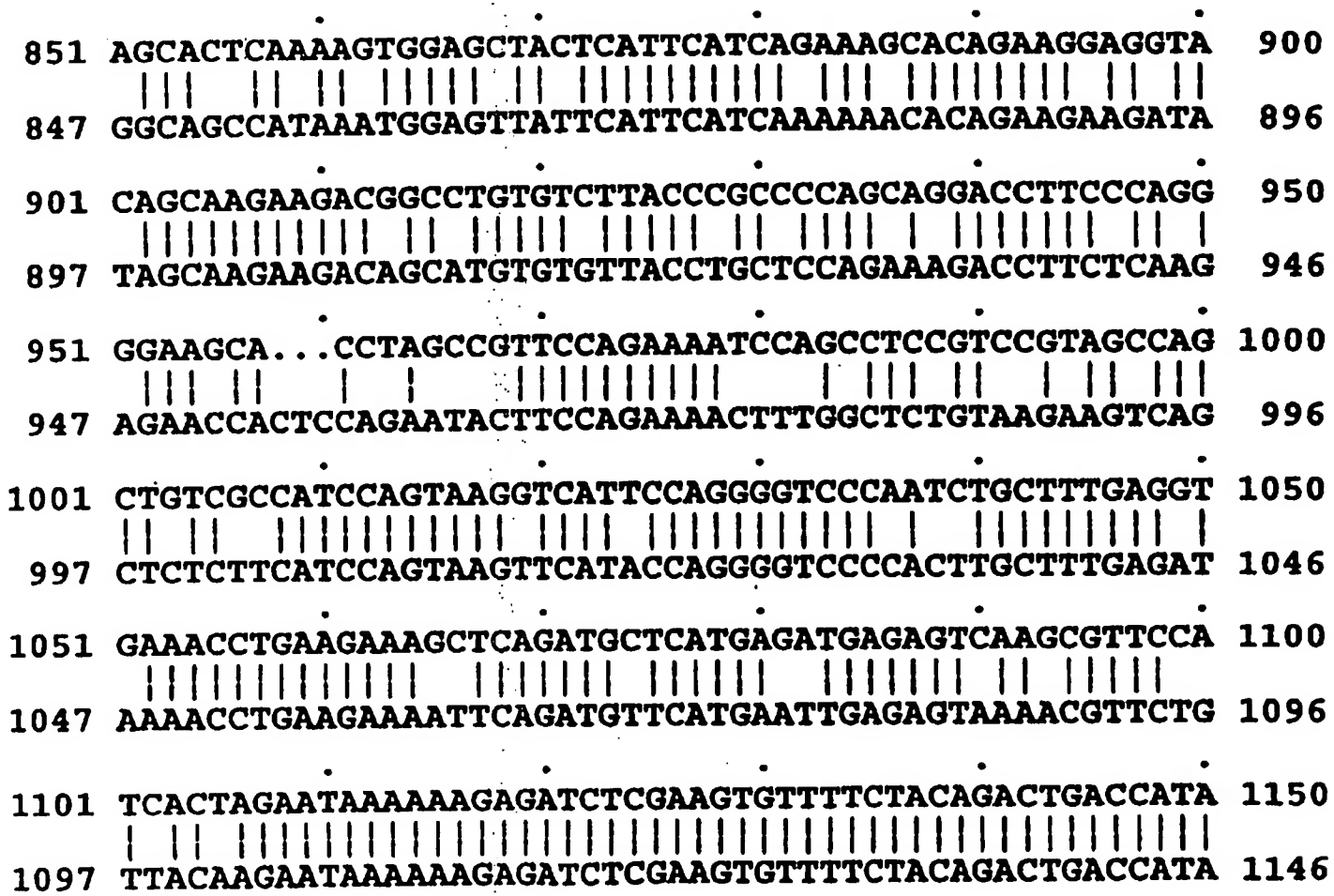

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FIGURE 7E

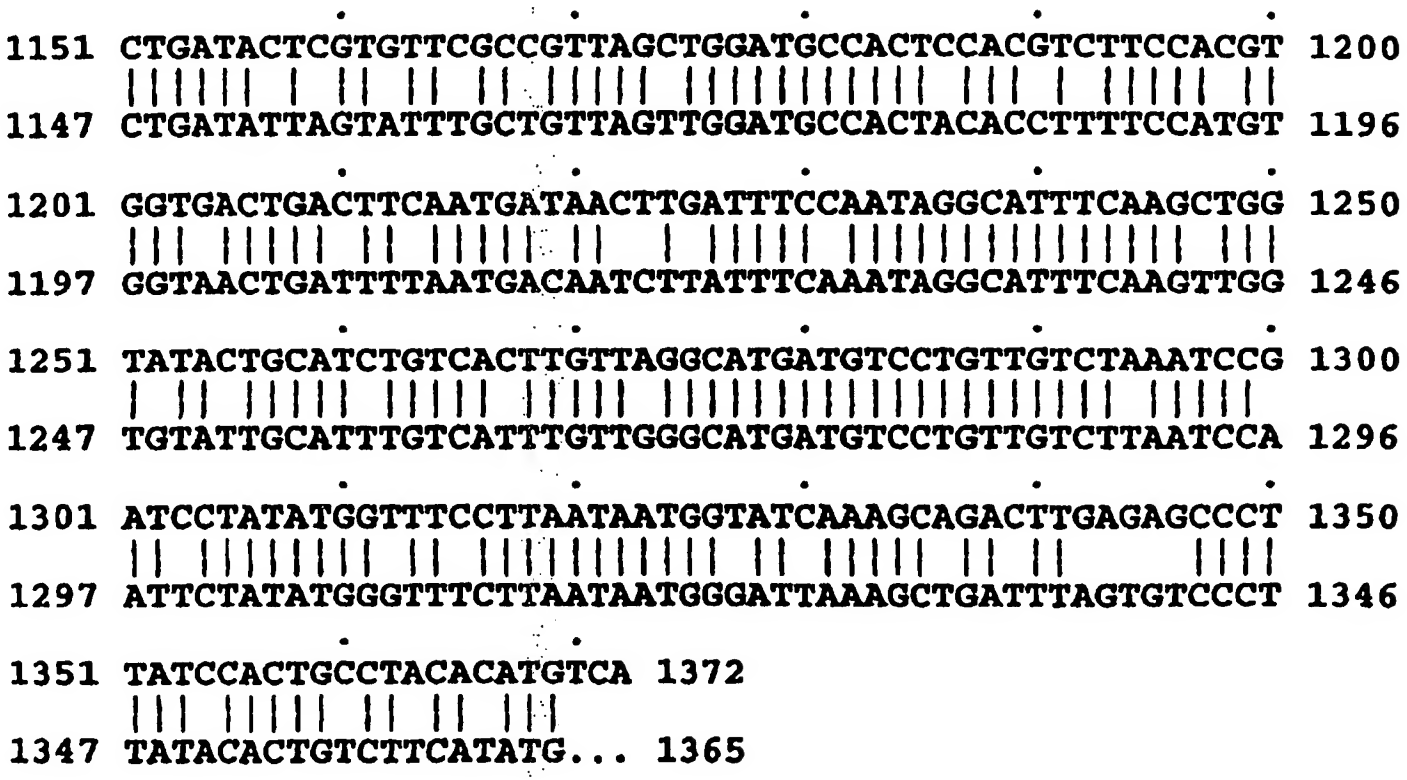

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FIGURE 7F

FIGURE 7F
FIGURE 7G

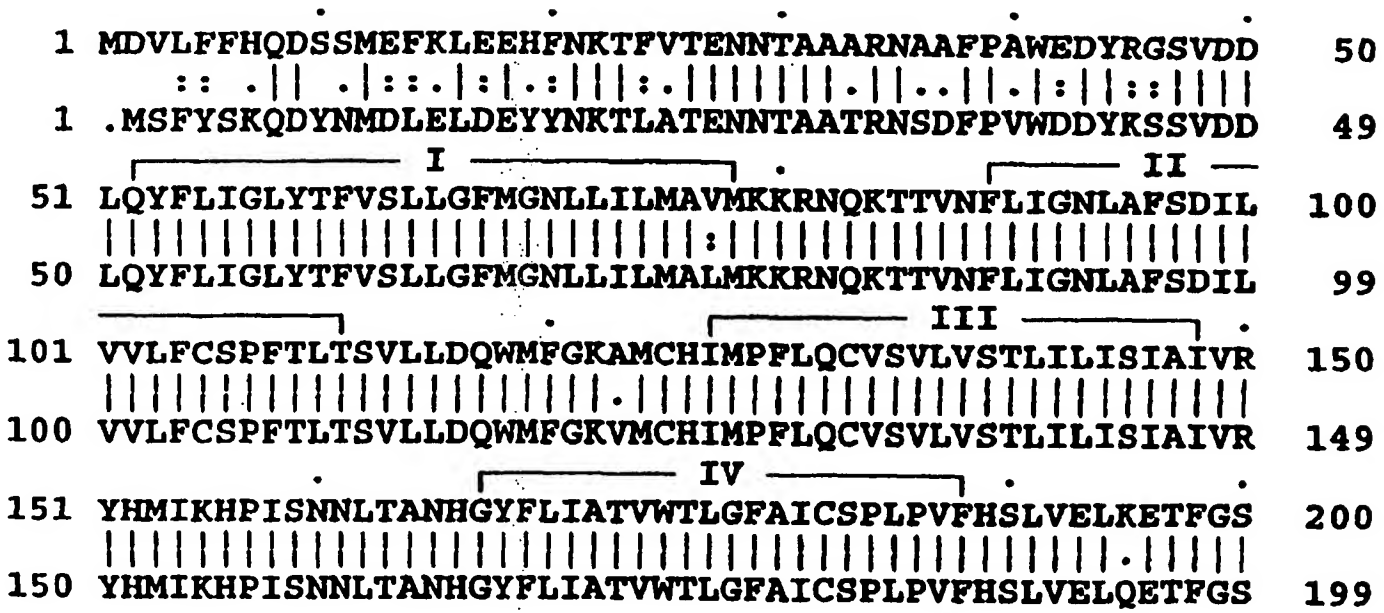

FIGURE 7G

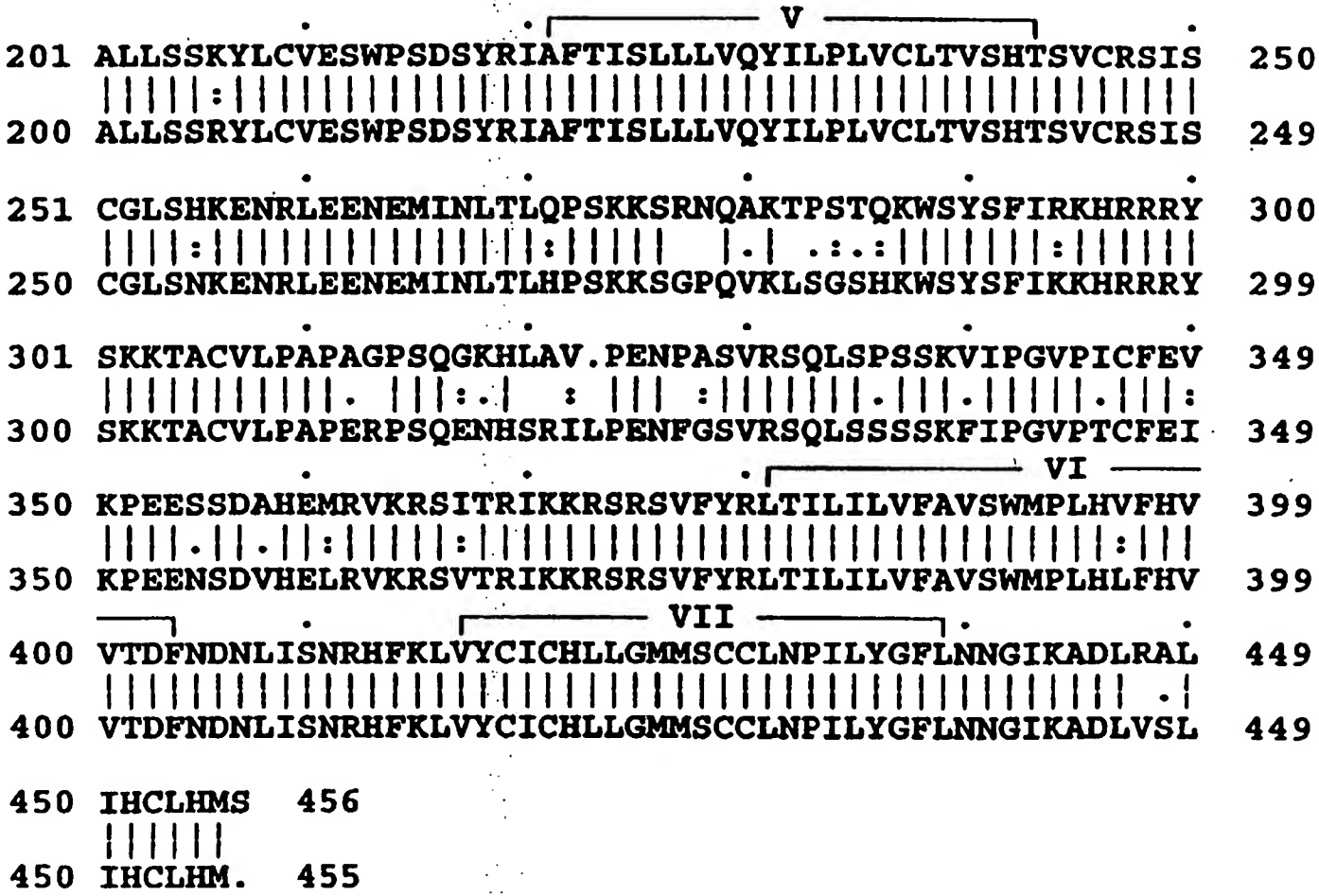

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FIGURE 8A

FIGURE 8A
FIGURE 8B
FIGURE 8C

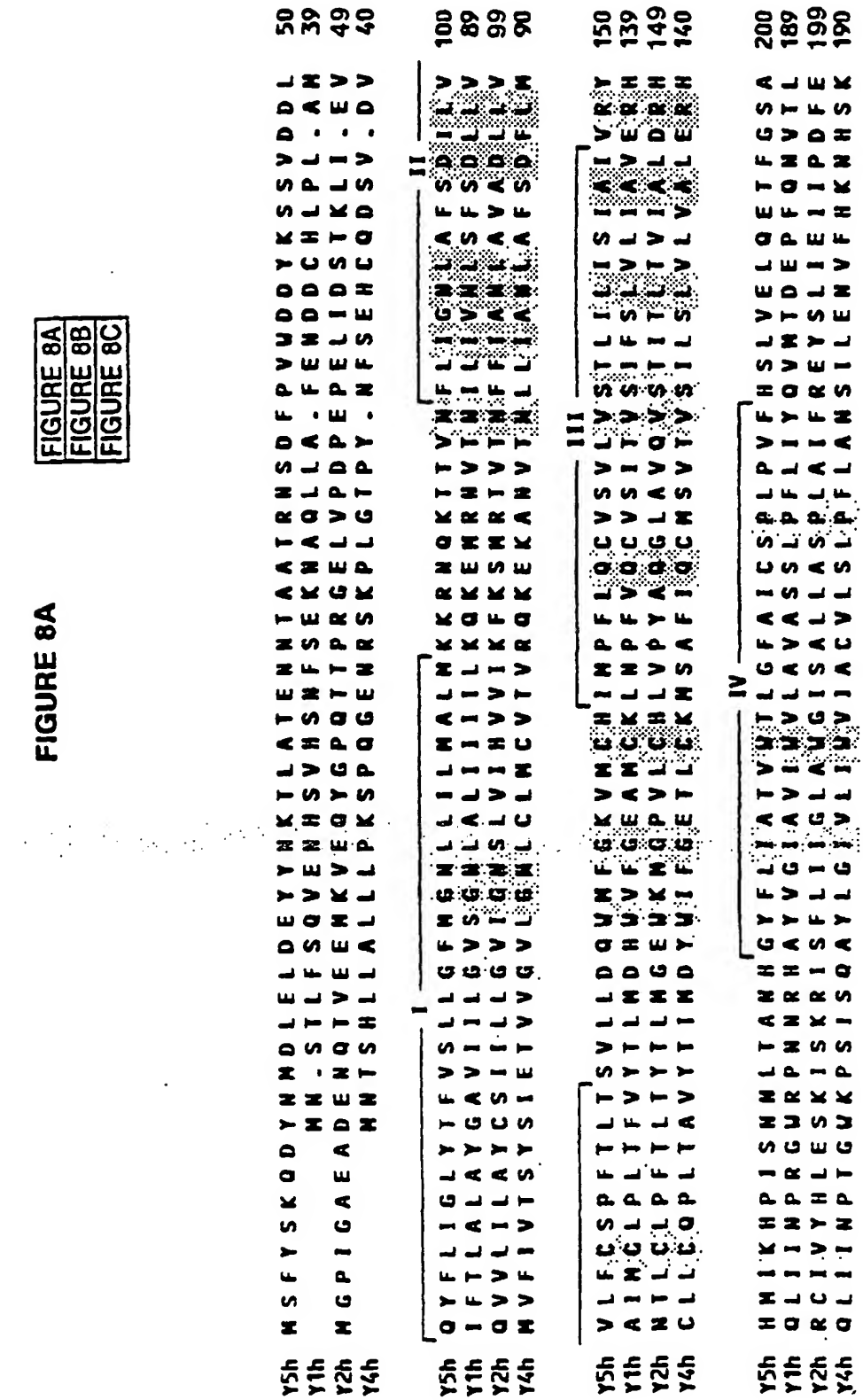

FIGURE 8B

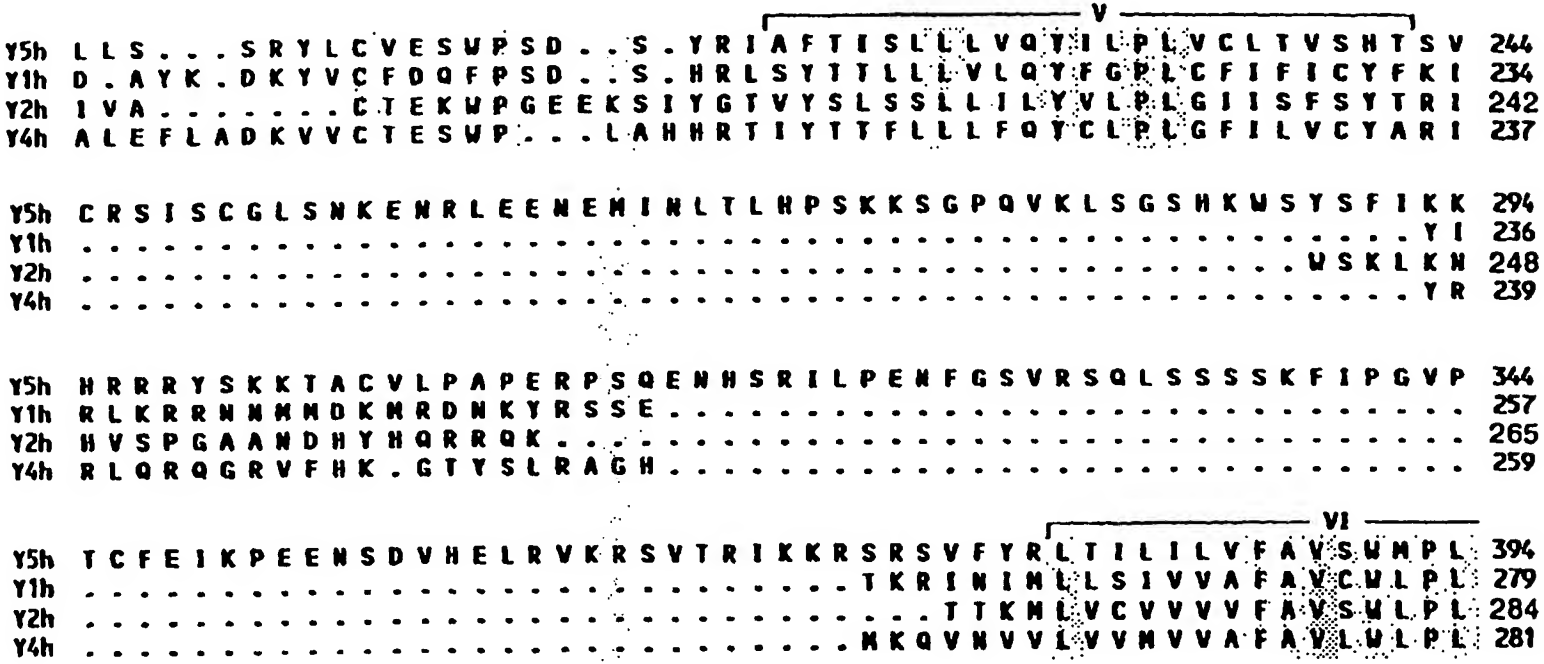
V
Y5h LLS...SRYLCVESWPSD..S.YRIAFTISLLLVQTILPLVCLTVSHTSV 244
Y1h D.AYK.DKYVCFDQFPSD..S.HRLSYTTLLLVLQYFGPLCFIFICYFKI 234
Y2h IVA.......CTEKWPGEEKSIYGTVYSLSSLLILYVLPLGIISFSYTRI 242
Y4h ALEFLADKVVCTESWP...LAHHRTIYTTFLLLFQYCLPLGFILVCYARI 237
Y5h CRSISCGLSNKENRLEENEMINLTLHPSKKSGPQVKLSGSHKWSYSFIKK 294
Y1h ....YI 236
Y2h ....WSKLKN 248
Y4h ....YR 239
Y5h HRRRYSKKTACVLPAPERPSQENHSRILPENFGSVRSQLSSSSKFIPGVP 344
Y1h RLKRRNNMMDKMRDNKYRSSE..... 257
Y2h HVSPGAANDHYHQRRQK..... 265
Y4h RLQRQGRVFHK.GTYSLRAGH..... 259
VI
Y5h TCFEIKPEENSDVHELRVKRSVTRIKKRSRSVFYRLTILILVFAVSWMPL 394
Y1h ..........TKRINIMLLSIVVAFAVCWLPL 279
Y2h ..........TTKMLVCVVVVFAVSWLPL 284
Y4h ..........MKQVNVVLVVMVVAFAVLWLPL 281

FIGURE 8C

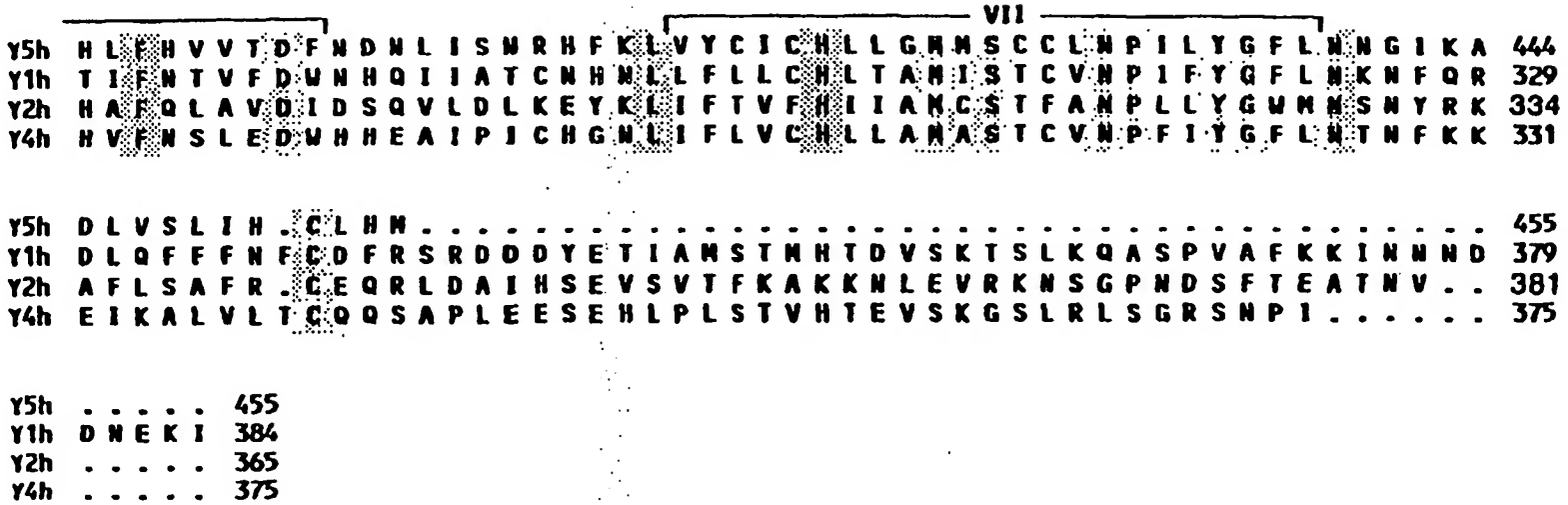
VII
Y5h HLFHVVTDFNDNLISNRHFKLVYCICHLLGMMSCCLNPILYGFLNNGIKA 444
Y1h TIFNTVFDWNHQIIATCNHNLLFLLCHLTAMISTCVNPIFYGFLNKNFQR 329
Y2h HAFQLAVDIDSQVLDLKEYKLIFTVFHIIAMCSTFANPLLYGWMNSNYRK 334
Y4h HVFNSLEDWHHEAIPICHGNLIFLVCHLLAMASTCVNPFIYGFLNTNFKK 331
Y5h DLVSLIH.CLHM..... 455
Y1h DLQFFFNFCDFRSRDDDYETIAMSTMHTDVSKTSLKQASPVAFKKINNNND 379
Y2h AFLSAFR.CEQRLDAIHSEVSVTFKAKKNLEVRKNSGPNDSFTEATNV.. 381
Y4h EIKALVLTCQQSAPLEESEHLPLSTVHTEVSKGSLRLSGRSNPI..... 375
Y5h 455
Y1h DNEKI 384
Y2h 365
Y4h 375

FIGURE 9

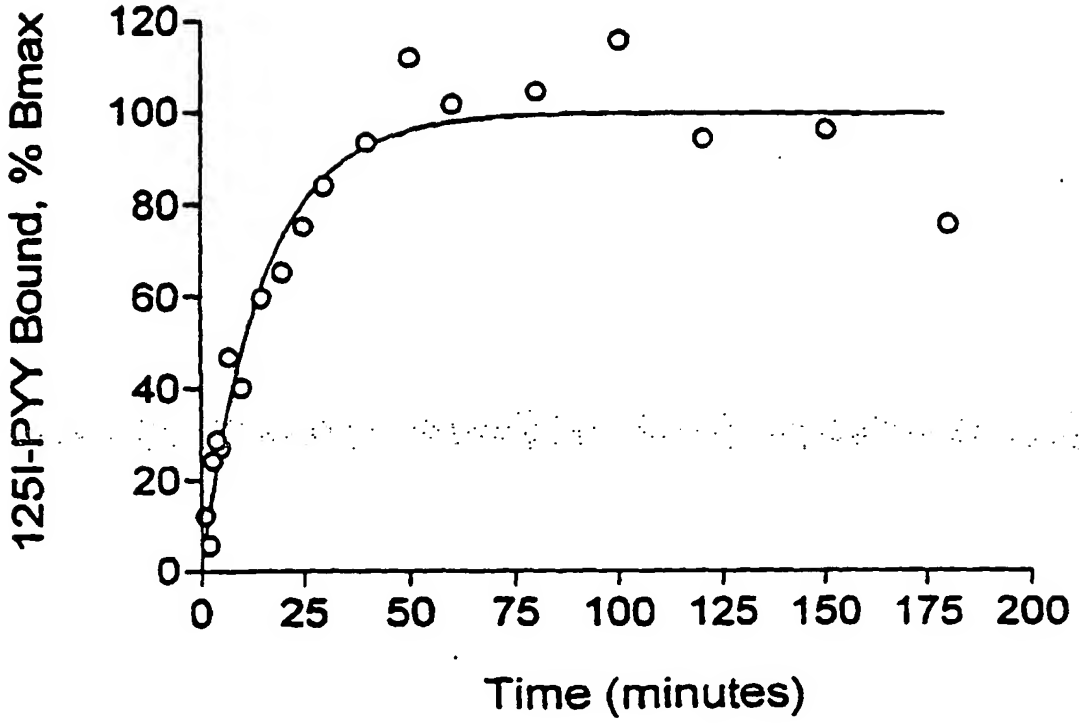
125I-PYY Bound, % Bmax
120
100
80
60
40
20
0
0
25
50
75
100
125
150
175
200
Time (minutes)

FIGURE 10

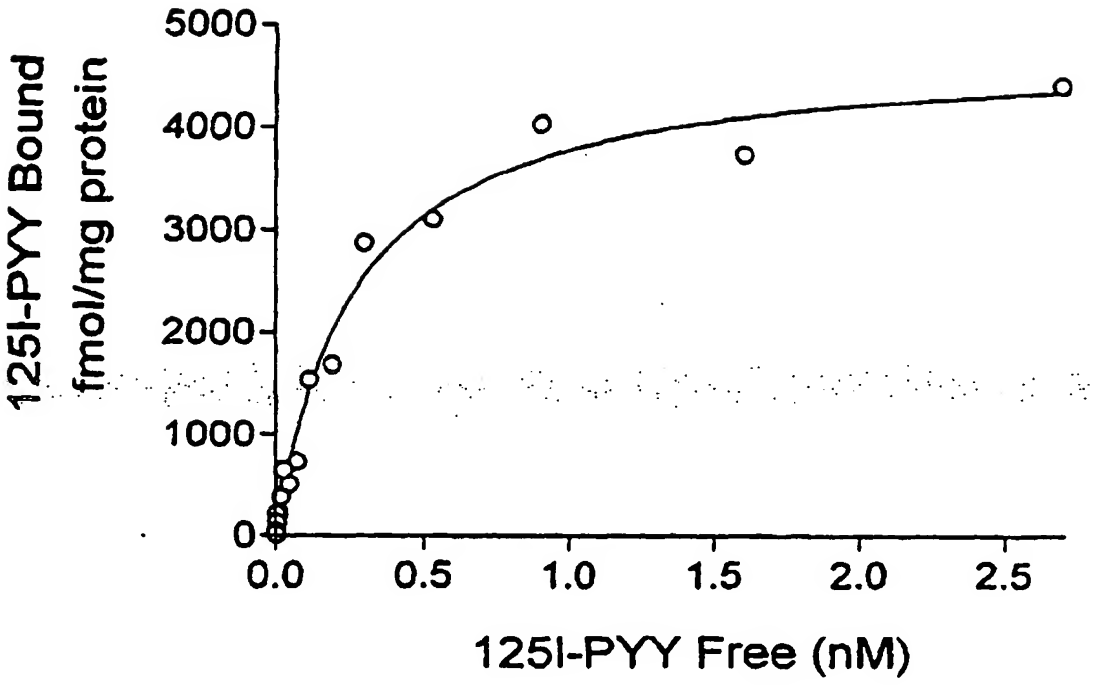
125I-PYY Bound
fmol/mg protein
5000
4000
3000
2000
1000
0
0.0
0.5
1.0
1.5
2.0
2.5
125I-PYY Free (nM)

FIGURE 11

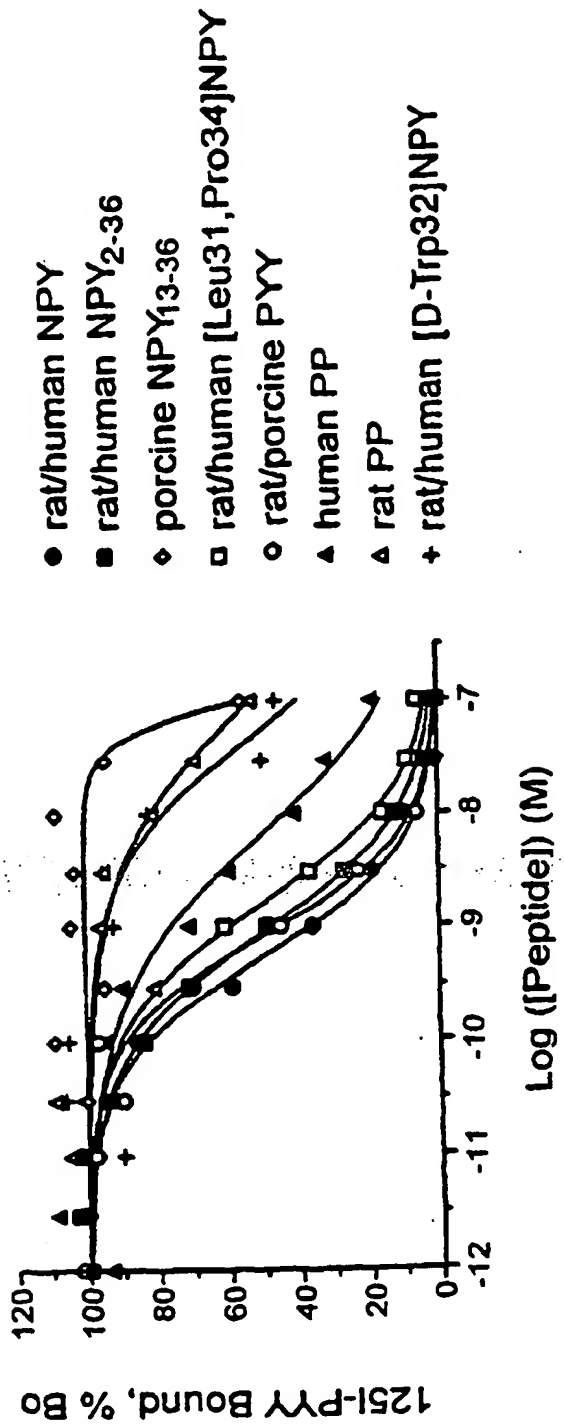
● rat/human NPY
■ rat/human NPY2-36
◆ porcine NPY13-36
□ rat/human [Leu31,Pro34]NPY
○ rat/porcine PYY
▲ human PP
△ rat PP
+ rat/human [D-Trp32]NPY
125I-PYY Bound, % Bo
120
100
80
60
40
20
0
-12
-11
-10
-9
-8
-7
Log ([Peptide]) (M)

FIGURE 12

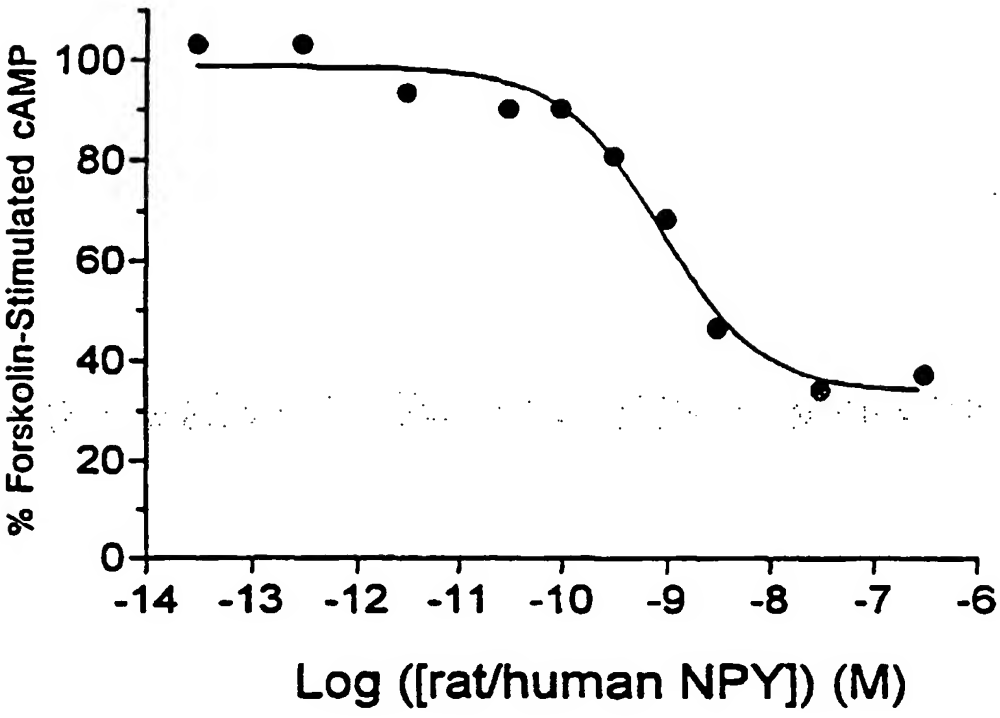
% Forskolin-Stimulated cAMP
100
80
60
40
20
0
-14
-13
-12
-11
-10
-9
-8
-7
-6
Log ([rat/human NPY]) (M)

FIGURE 13A

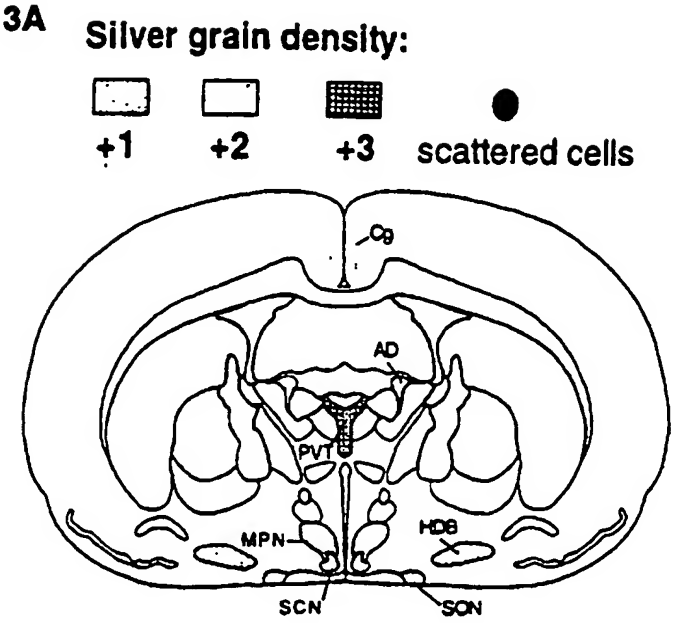

FIGURE 13B

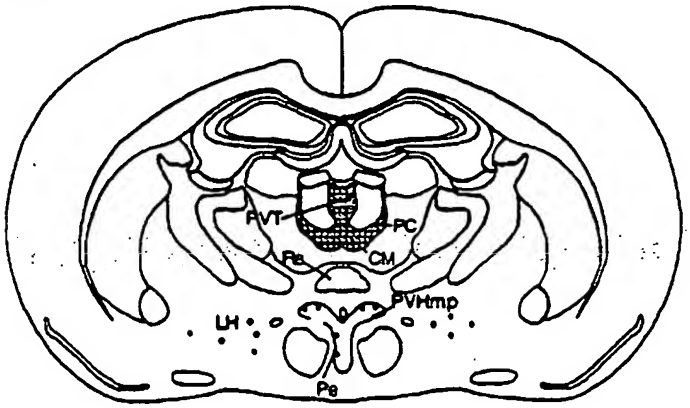

FIGURE 13C

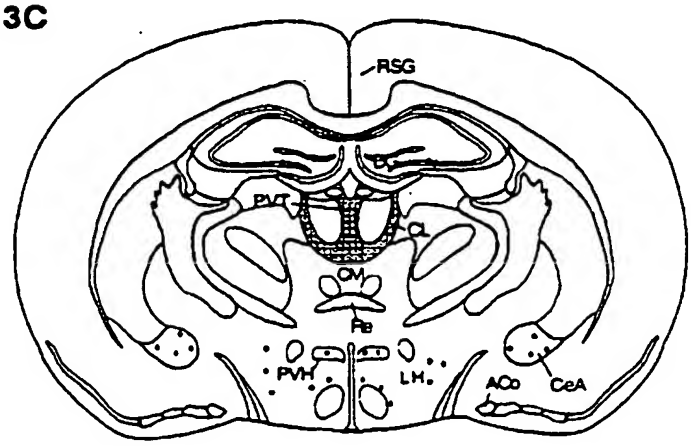

FIGURE 13D

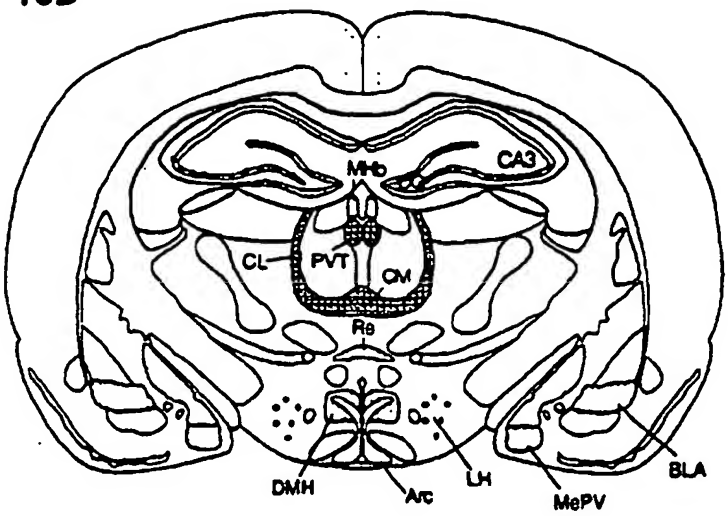

FIGURE 13E

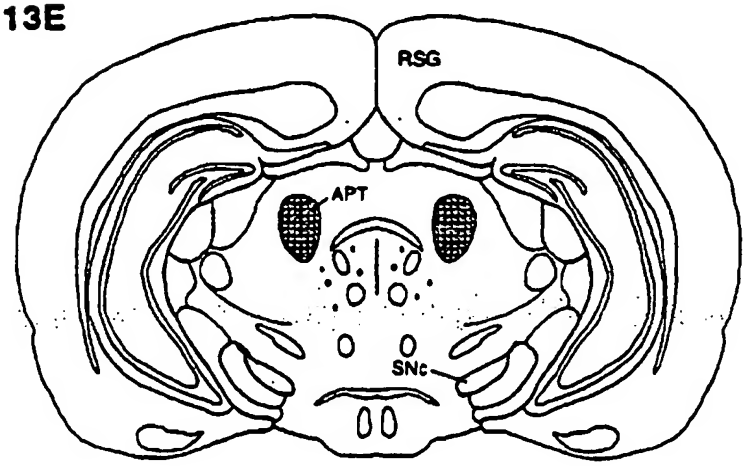

FIGURE 13F

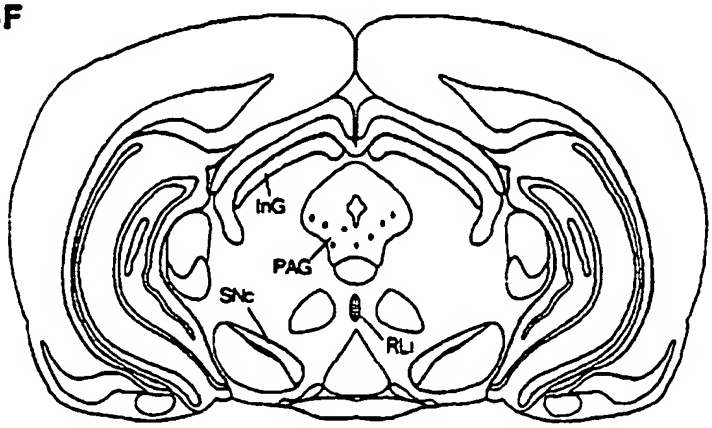

FIGURE 13G

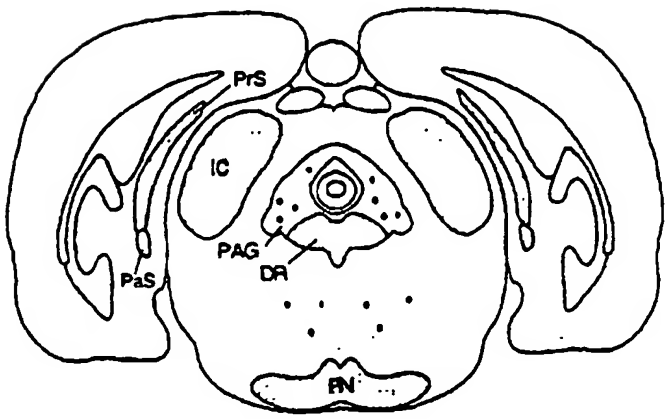

FIGURE 13H

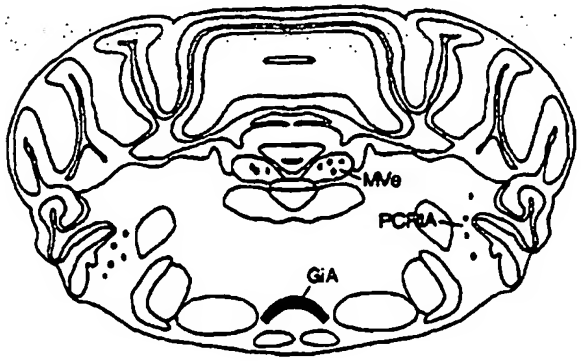

FIGURE 14

```
   1  TCATGTGTCA CATTATGCCT TTTCTTCAAT GTGTGTCAGT TCTGGTTTCA   50
  51  ACTTTAATTC TAATATCAAT TGCCATTGTC AGGTATCATA TGATCAAGCA   51
 101  TCCTATATCT AACAATTTAA CAGCAAACCA TGGCTACTTC CTGATTGCTA  150
 151  CTGTCTGGAC ACTAGGTTTT GCGATTTGTT CTCCCCTTCC AGTGTTTCAC  200
 201  AGTCTGGTGG AACTTCAGGA AACATTTGAC TCCGCATTGC TGAGCAGCAG  250
 251  GTATTTATGT GTTGAGTCGT GGCCATCTGA TTCGTACAGA ATCGCTTTTA  300
 301  CTATCTCTTT ATTGCTAGTC CAGTATATTC TTCCCTTGGT GTGTCTAACT  350
 351  GTGAGCCATA CCAGTGTCTG CAGGAGTATA AGCTGCGGGT TGTCCAACAA  400
 401  AGAAAACAAA CTGGAAGAAA ACGAGATGAT CAACTTAACT CTTCAACCAT  450
 451  TCAAAAAGAG TGGGCCTCAG GTGAAACTTT CCAGCAGCCA TAAATGGAGC  500
 501  TATTCATTCA TCAGAAAACA CAGGAGAAGG TACAGCAAGA AGACGGCGTG  550
 551  TGTCTTACCT GCTCCAGCAA GACCTCCTCA AGAGAACCAC TCAAGAATGC  600
 601  TTCCAGAAAA CTTTGGTTCT GTAAGAAGTC AGCATTCTTC ATCCAGTAAG  650
 651  TTCATACCGG GGGTCCCCAC CTGCTTTGAG GTGAAACCTG AAGAAAACTC  700
 701  GGATGTTCAT GACATGAGAG TAAACCGTTC TATCATGAGA ATCAAAAAGA  750
 751  GATCCCGAAG TGTTTTCTAT AGACTAACCA TACTGATACT AGTGTTTGCC  800
 801  GTTAGCTGGA TGCCACTACA CCTTTTCCAT GTGGTAACTG ATTTTAATGA  850
 851  CAACCTCATT TCAAACAGGC ATTTCAAATT GGTGTATTGC ATTTGTCATT  900
 901  TGTTAGGCAT GATGTCCTGT TGTCTTAATC CTATTCTGTA TGGTTTTCTC  950
 951  AATAATGGGA TCAAAGCTGA TTTAATTTCC CTTATACAGT GTCTTCATAT 1000
1001  GTCATAATTA TTAATGTTTA CCAAGGAGAC AACAAATGTT GGGATCGTCT 1050
1051  AAAA
```

FIGURE 15

```
  1  MCHIMPFLQC VSVLVSTLIL ISIAIVRYHM IKHPISNNLT ANHGYFLIAT   50
 51  VWTLGFAICS PLPVFHSLVE LQETFDSALL SSRYLCVESW PSDSYRIAFT  100
101  ISLLLVQYIL PLVCLTVSHT SVCRSISCGL SNKENKLEEN EMINLTLQPF  150
151  KKSGPQVKLS SSHKWSYSFI RKHRRRYSKK TACVLPAPAR PPQENHSRML  200
201  PENFGSVRSQ HSSSSKFIPG VPTCFEVKPE ENSDVHDMRV NRSIMRIKKR  250
251  SRSVFYRLTI LILVFAVSWM PLHLFHVVTD FNDNLISNRH FKLVYCICHL  300
301  LGMMSCCLNP ILYGFLNNGI KADLISLIQC LHMS
```


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FIGURE 16A

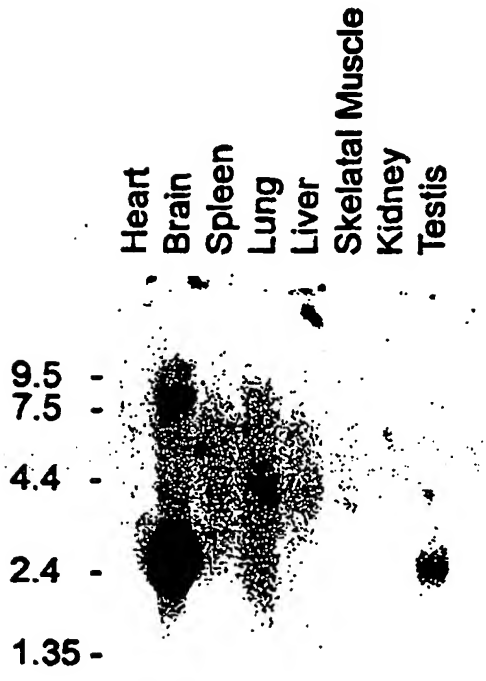
Heart
Brain
Spleen
Lung
Liver
Skelatal Muscle
Kidney
Testis
9.5
7.5
4.4
2.4
1.35

FIGURE 16B

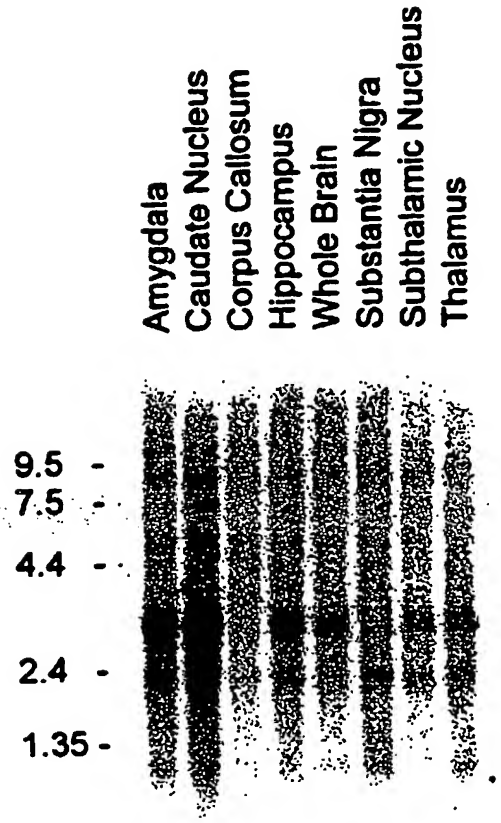
Amygdala
Caudate Nucleus
Corpus Callosum
Hippocampus
Whole Brain
Substantia Nigra
Subthalamic Nucleus
Thalamus
9.5
7.5
4.4
2.4
1.35

FIGURE 16C

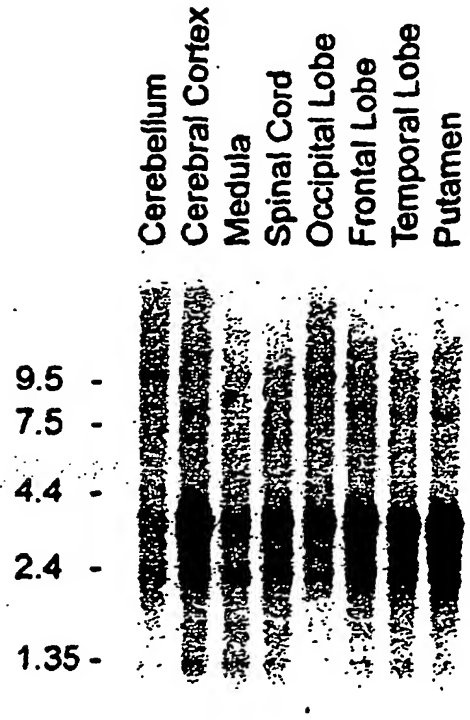
Cerebellum
Cerebral Cortex
Medula
Spinal Cord
Occipital Lobe
Frontal Lobe
Temporal Lobe
Putamen
9.5
7.5
4.4
2.4
1.35

FIGURE 17A

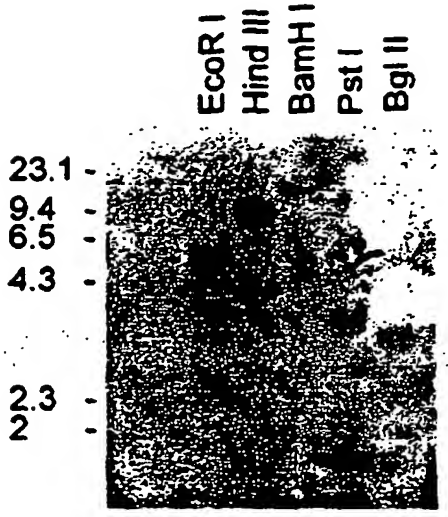

FIGURE 17B

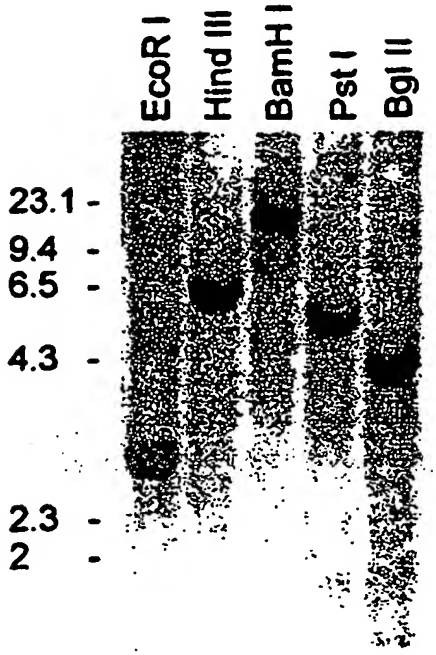

FIGURE 18

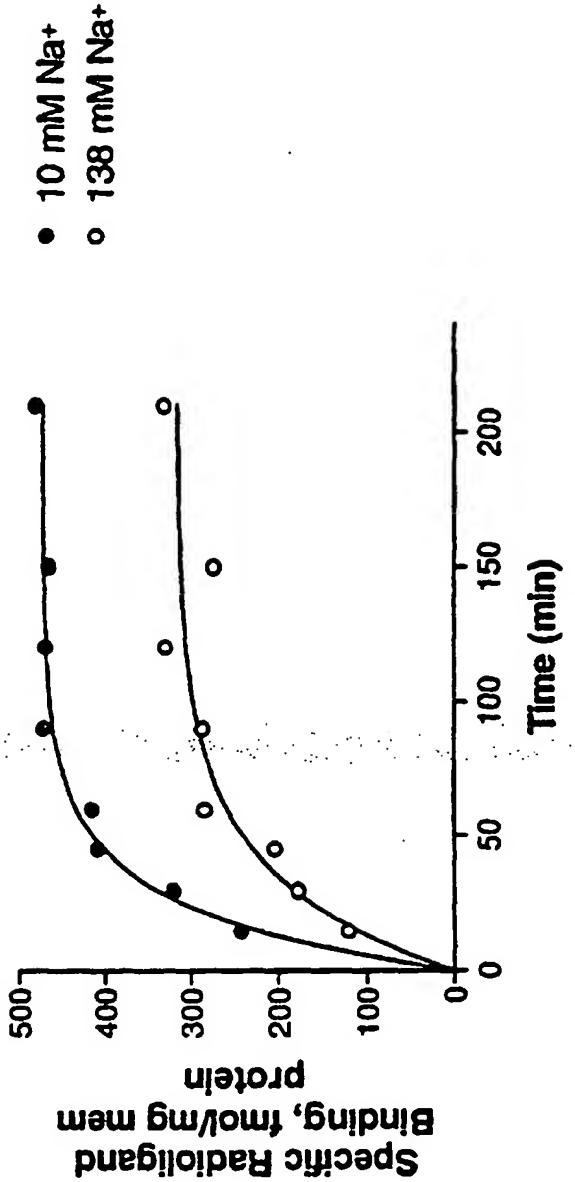
• 10 mM Na+
o 138 mM Na+
Specific Radioligand Binding, fmol/mg mem protein
500
400
300
200
100
0
0
50
100
150
200
Time (min)

FIGURE 19

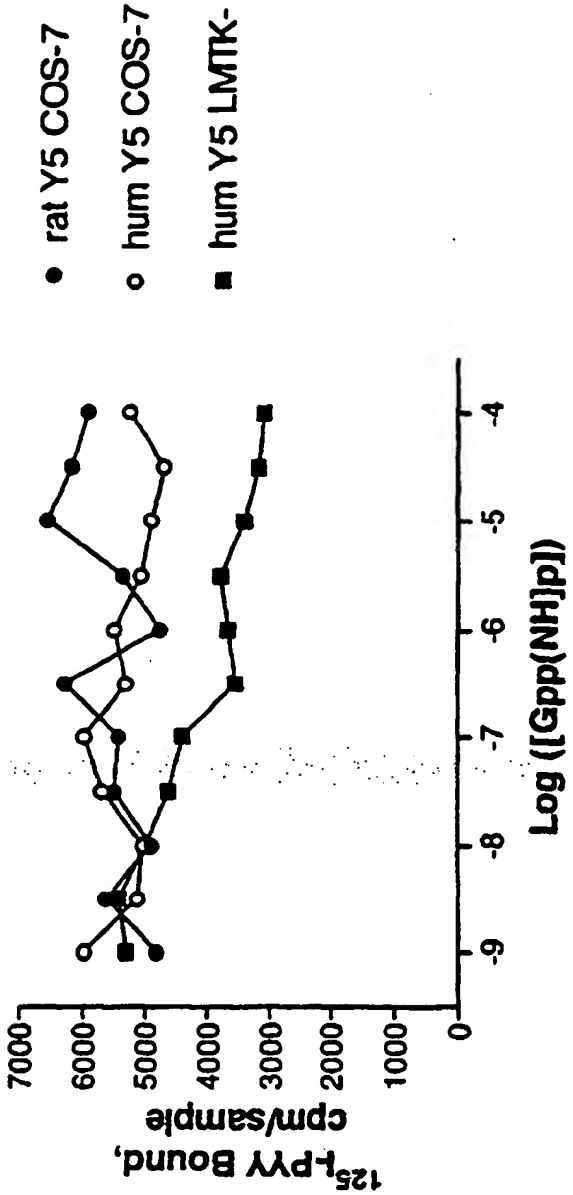
• rat Y5 COS-7
o hum Y5 COS-7
■ hum Y5 LMTK-
^{125}I-PYY Bound, cpm/sample
7000
6000
5000
4000
3000
2000
1000
0
-9
-8
-7
-6
-5
-4
Log ([Gpp(NH)p])

FIGURE 20

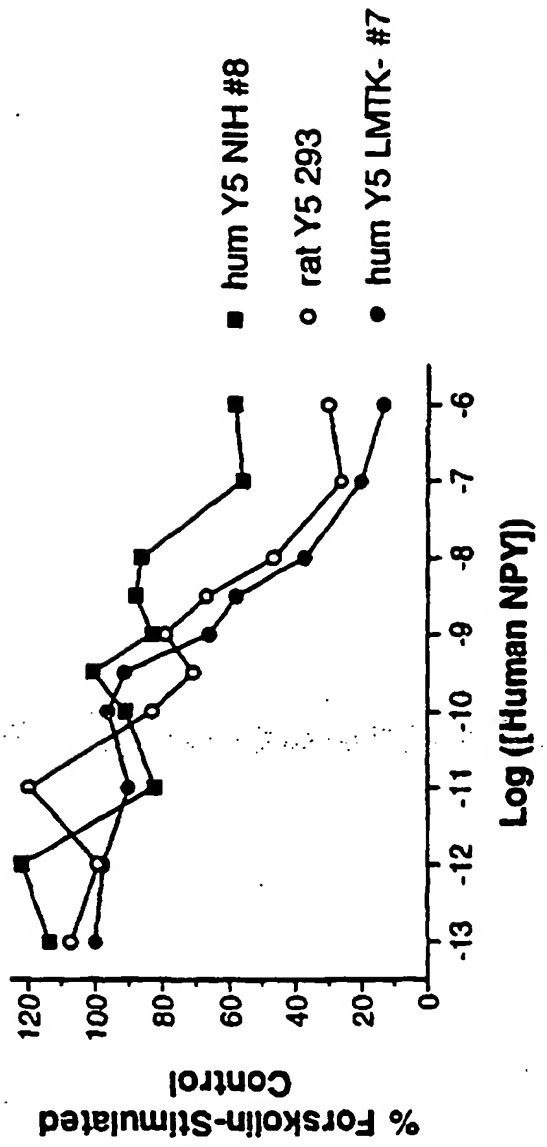
% Forskolin-Stimulated Control
120
100
80
60
40
20
0
-13
-12
-11
-10
-9
-8
-7
-6
Log ([Human NPY])
hum Y5 NIH #8
rat Y5 293
hum Y5 LMTK- #7

FIGURE 21A

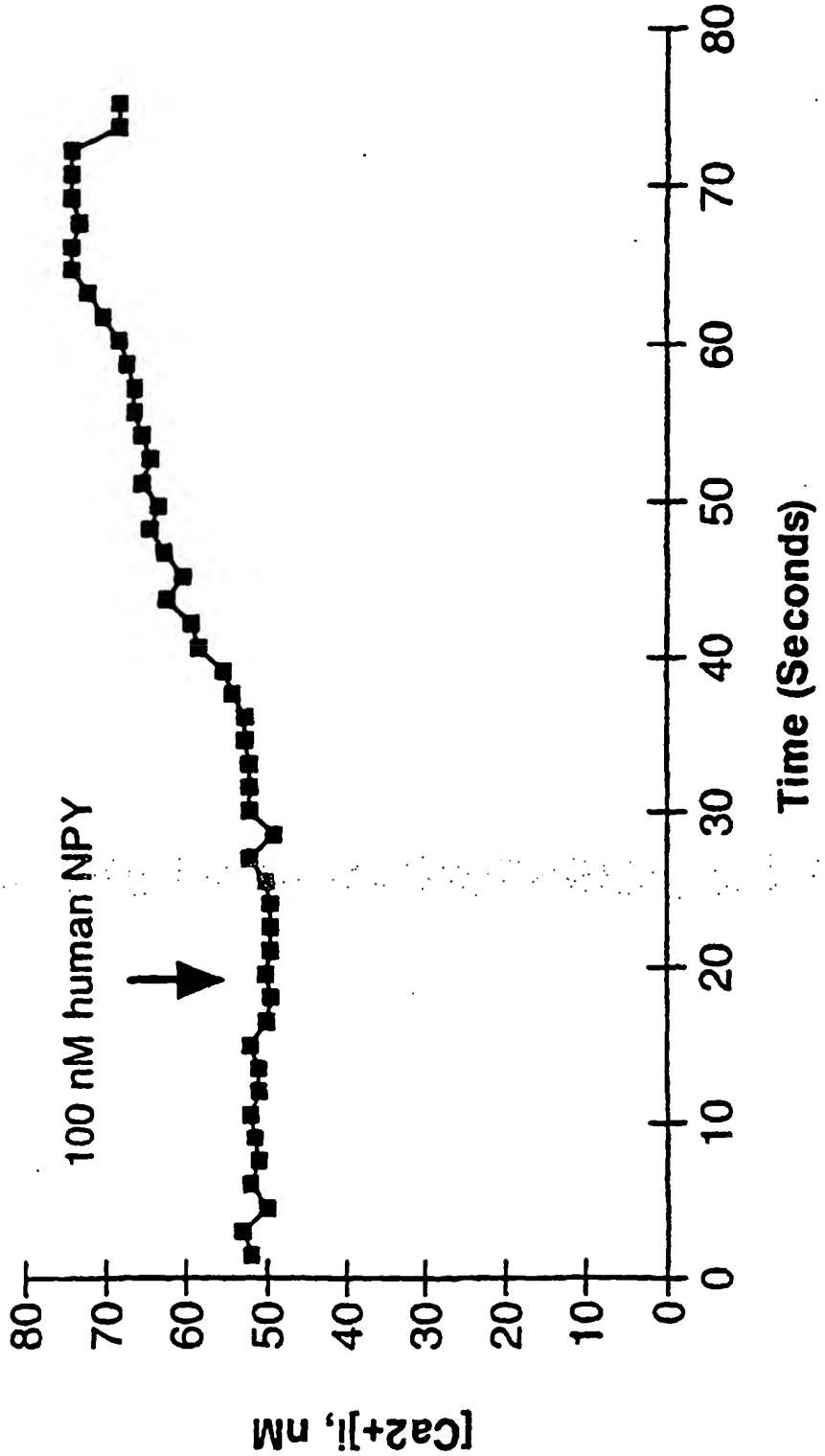
[Ca2+]i, nM
0
10
20
30
40
50
60
70
80
100 nM human NPY
0
10
20
30
40
50
60
70
80
Time (Seconds)

FIGURE 21B

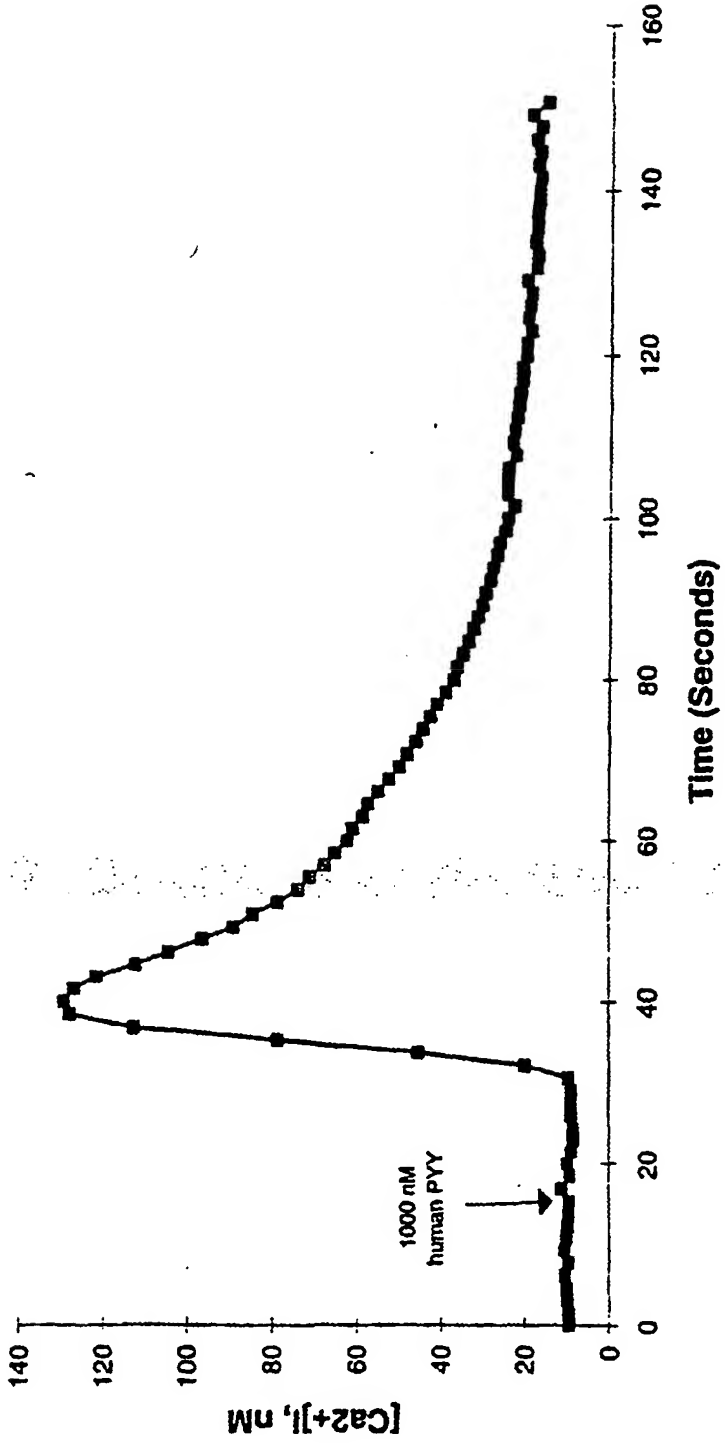
[Ca2+]i, nM
0
20
40
60
80
100
120
140
1000 nM
human PYY
0
20
40
60
80
100
120
140
160
Time (Seconds)

FIGURE 21C

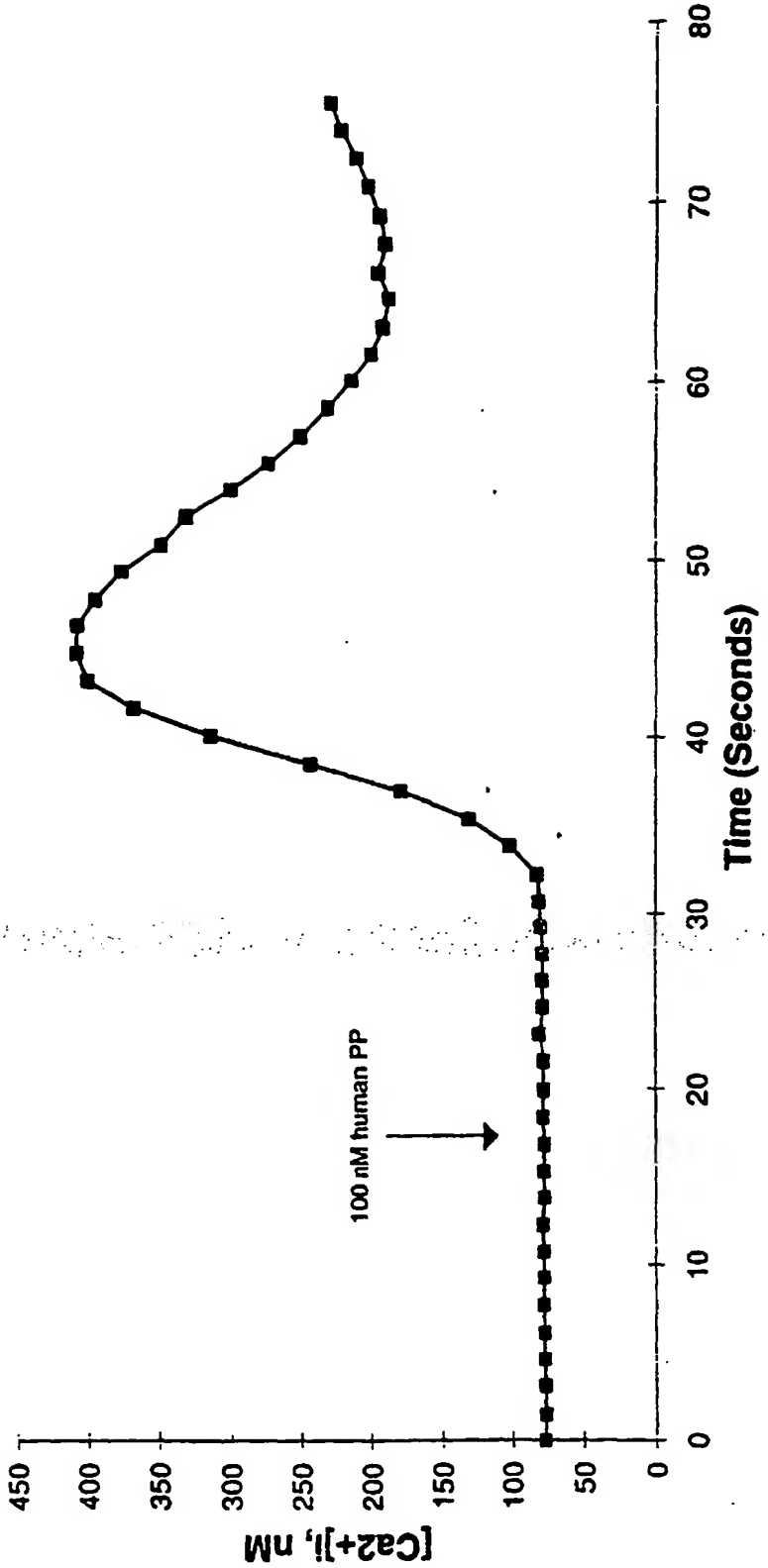
[Ca2+]i, nM
450
400
350
300
250
200
150
100
50
0
0
10
20
30
40
50
60
70
80
Time (Seconds)
100 nM human PP

FIGURE 21D

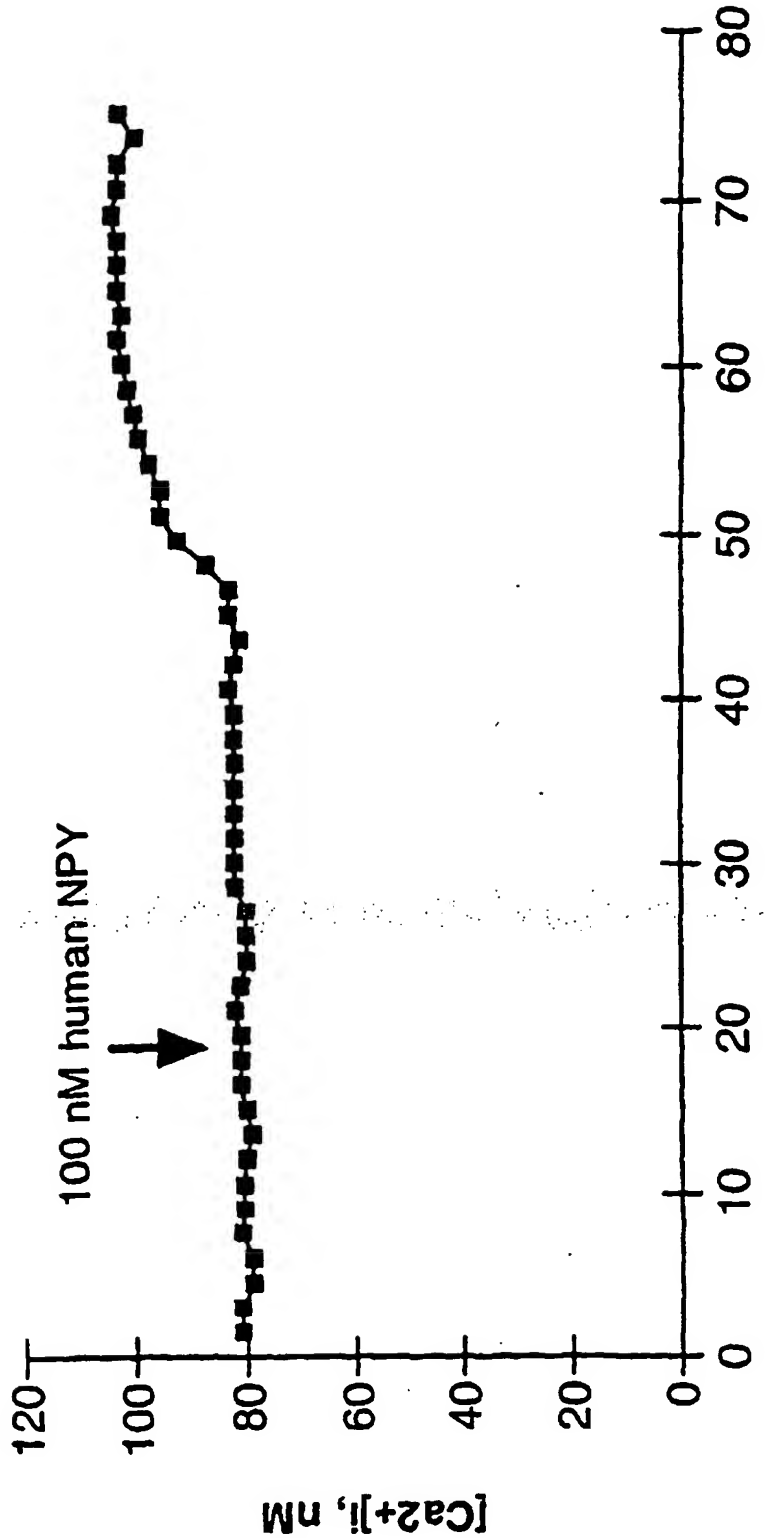
[Ca2+]i, nM
120
100
80
60
40
20
0
0
10
20
30
40
50
60
70
80
Time (Seconds)
100 nM human NPY

FIGURE 22